jc760 U.S. PTO
01/17/00

PATENT

Docket No. 2026-4149US4
Express Mail Label No. EJ604726585US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

UTILITY APPLICATION AND FEE TRANSMITTAL (1.53(b))

jc688 U.S. PTO
09/483831
01/17/00

ASSISTANT COMMISSIONER FOR PATENTS
Box Patent Application
Washington, D.C. 20231

Sir:

Transmitted herewith for filing is the patent application of

First named Inventor
or Application Identifier: Stracke, M. et al.

For: AUTOTAXIN: MOTILITY STIMULATING PROTEIN
USEFUL IN CANCER DIAGNOSIS AND THERAPY

Enclosed are

[X] 47 page(s) of specification, 1 page(s) of Abstract, 3 page(s) of claims (as originally filed in the parent application)

[X] 19 sheets of drawing (Figs and) [ ] formal [x] informal (as originally filed in the parent application)

[X] 4 page(s) of Declaration and Power of Attorney

[ ] Unsigned
[ ] Newly Executed
[x] Copy from prior application

[ ] Deletion of inventors including Signed Statement under 37 C.F.R. § 1.63(d)(2)

[ ] ___ page(s) of Revocation of Power of Attorney and New Power of Attorney

[X] Please direct all correspondence and all telephone calls in this matter to:
William S. Feiler (Reg. 26,728)
Morgan & Finnegan, LLP
345 Park Avenue
New York, NY 10154
(212) 758-4800

[x] Incorporation by Reference: The entire disclosure of the prior application, from which a copy of the combined declaration and power of attorney is supplied herein, is considered as being part of the disclosure of the accompanying application and is incorporated herein by reference.

[ ] Microfiche Computer Program (Appendix)

[X] 46 page(s) of Sequence Listing

[x] computer readable disk containing Sequence Listing

[x] Statement under 37 C.F.R. § 1.821(f) that computer and paper copies of the Sequence Listing are the same

[ ] Certified copy of Priority Document(s)

[ ] English translation documents

[X] Information Disclosure Statement

[ ] Copy of ___ cited references

[X] Preliminary Amendment

[X] Return receipt postcard (MPEP 503)

[X] Assignment Papers (assignment cover sheet and assignment documents)

[ ] A check in the amount of $40.00 for recording the Assignment.

[x] Assignment papers as filed in application Serial No. 08/346,455, recorded on Reel 7324, Frame 0799 on February 10, 1995.

[ ] Certification of chain of title pursuant to 37 C.F.R. § 3.73(b).

[X] This is a [X] continuation [ ] divisional [ ] continuation-in-part (C-I-P) of prior application serial no. 08/977,221, herein incorporated by reference.

[x] Cancel in this application original claims 1-19 of the parent application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

[x] A Preliminary Amendment is enclosed. (Claims added by this Amendment have been properly numbered consecutively beginning with the number following the highest numbered original claim in the prior application.

[X] The status of the parent application is as follows:

[ ] A Petition For Extension of Time and a Fee therefor has been or is being filed in the parent application to extend the term for action in the parent application until _____.

[ ] A copy of the Petition for Extension of Time in the co-pending parent application is attached.

[x] No Petition For Extension of Time and Fee therefor are necessary in the co-pending parent application.

[ ] Please abandon the parent application at a time while the parent application is pending or at a time when the petition for extension of time in that application is granted and while this application is pending has been granted a filing date, so as to make this application co-pending.

[ ] Transfer the drawing(s) from the parent application to this application.

[X] Amend the specification by inserting before the first line the sentence:
This is a [X] continuation [ ] divisional [ ] continuation-in-part of co-pending application Serial No. 08/977,221 filed November 24, 1997, herein incorporated by reference.

I. CALCULATION OF APPLICATION FEE

	Number Filed	Number Extra	Rate		Basic Fee
					$690.00
Total Claims	6	-20= 0	x	$18.00	$
Independent Claims	1	- 3= 0	x	$78.00	$
Multiple Dependent Claims					
	[X] yes Additional fee	=	$260.00	$	$260.00
	[ ] no Additional fee	=	NONE		
				Total:	$950.00

[ ] A statement claiming small entity status is attached or has been filed in the above-identified parent application and its benefit under 37 C.F.R. § 1.28(a) is hereby claimed. Reduced fees under 37 C.F.R. § 1.9(F) (50% of total) paid herewith $_____.

[X] A check in the amount of $ 950.00 in payment of the application filing fees is attached.

[X] Charge Fee(s) to Deposit Account No. 13-4500. Order No. 2026-4149US4 . A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

[X] The Assistant Commissioner is hereby authorized to charge any additional fees which may be required for filing this application, or credit any overpayment to Deposit Account No. 13-4500 Order No. 2026-4149US4. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

Respectfully submitted,

MORGAN & FINNEGAN, L.L.P.

Dated: January 17, 2000

By: _____
Dorothy R. Auth
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01-19-00

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PATENT
Docket No. 2026-4149US4

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Box Sec

Applicant(s) : Stracke, M. et al. Group Art Unit: To Be Assigned

Continuation Application
of Serial No. : 08/977,221 Examiner: To Be Assigned

Filed : Herewith

For : AUTOTAXIN: MOTILITY STIMULATING PROTEIN USEFUL IN CANCER DIAGNOSIS AND THERAPY

EXPRESS MAIL CERTIFICATE

Express Mail Label No. EJ604726585US

Date of Deposit January 17, 2000

I hereby certify that the following attached paper(s) or fee

1. Utility Application and Fee Transmittal (1.53(b));
2. Copy of Patent Application (including 47 pages of specification; 3 pages of claims, 1 page of abstract and 19 pages of drawings);
3. Copy of Combined Declaration and Power of Attorney;
4. Copy of Associate Power of Attorney;
5. Copy of Assignment;
6. Paper Copy of Sequence Listing (46 pages);
7. 1 computer disk containing Sequence Listing;
8. Statement Under 37 C.F.R. §1.821(f);
9. Copy of PTO-1449 (9 sheets) and ;
10. Return Postcard.

is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. §1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, BOX PATENT APPLICATION, Washington, D.C. 20231.

Francisco J. Garcia
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PATENT
Docket No. 2026-4149US4

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Stracke, M. et al. Group Art unit : 1652

Continuation Application
of Serial No. : 08/977,221 Examiner : Longton, E.

Filed : Herewith

For : AUTOTAXIN: MOTILITY STIMULATING PROTEIN USEFUL IN CANCER DIAGNOSIS AND THERAPY

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
Washington, D.C. 20231

Sir:

Prior to examination and calculation of the filing fee, please amend the application as follows.

IN THE CLAIMS

Please cancel claims 1-19 and add the following new claims:

20. An isolated polypeptide comprising an amino acid sequence of human autotaxin, wherein said polypeptide thereof has cell motility activity.

21. The polypeptide of claim 20, wherein said polypeptide is a fragment thereof having at least 5 amino acids.

22. The polypeptide of claim 20 or 21, wherein said polypeptide is bound to a solid support.

23. A method of purifying the autotaxin polypeptide of claim 20 or 21, comprising the steps of:

i) collecting and concentrating supernatant from cultured A2058 human melanoma cells whereby a first preparation of said polypeptide is produced;

ii) salt fractionating said first preparation to produce a second polypeptide preparation;

iii) isolating said polypeptide from said second preparation so that said polypeptide is obtained in substantially pure form.

24. The method of claim 23, wherein said isolating step is effected by column chromatography.

25. A recombinant autotaxin polypeptide according to claim 20 or 21.

REMARKS

Applicants respectfully request favorable consideration of the present application and claims. Early and favorable action by the Examiner is earnestly solicited.

No additional fee is believed to be necessary.

The Commissioner is hereby authorized to charge any additional fees which may be required for this amendment, or credit any overpayment to Deposit Account No. 13-4500, Order No. 2026-4149US4.

In the event that an extension of time is required, or which may be required in addition to that requested in a petition and for an extension of time, the Commissioner is

requested to grant a petition for that extension of time which is required to make this response timely and is hereby authorized to charge any fee for such an extension of time or credit any overpayment for an extension of time to Deposit Account No. 13-4500, Order No. 2026-4149US4. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

Respectfully submitted,

MORGAN & FINNEGAN, L.L.P.

Dated: January 17, 2000

By: _____
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AUTOTAXIN: MOTILITY STIMULATING PROTEIN USEFUL IN CANCER DIAGNOSIS AND THERAPY

This application is a continuation-in-part of application serial no. 08/249,182 filed May 25, 1994, which is a continuation-in-part of application serial no. 07/822,043 filed on Jan. 17, 1992.

Field of the Invention

The present invention relates, in general, to a motility stimulating and compositions comprising the same. In particular, the present invention relates to a purified form of the protein and peptides thereof, for example, autotaxin (herein alternative referred to as "ATX"); a DNA segment encoding autotaxin; recombinant DNA molecules containing the DNA segment; cells containing the recombinant DNA molecule; a method of producing autotaxin; antibodies to autotaxin; and methods of cancer diagnosis and therapy using the above referenced protein or peptides thereof and DNA segments.

Background of the Invention

Cell motility plays an important role in embryonic events, adult tissue remodeling, wound healing, angiogenesis, immune defense, and metastasis of tumor cells (Singer, 1986). In normal physiologic processes, motility is tightly regulated. On the other hand, tumor cell motility may be aberrantly regulated or autoregulated. Tumor cells can respond in a motile fashion to a variety of agents. These include host-derived factors such as scatter factor (Rosen, et al., 1989) and growth factors (Kahan, et al., 1987; Stracke, et al.; Tamm, et al., 1989; Wang, et al. 1990; and Jouanneau, et al. 1991), components of the extracellular matrix (McCarthy, et al. 1984), and tumor-secreted or autocrine factors (Liotta, et al. 1988; Ruff, et al. 1985; Atnip, et al. 1987; Ohnishi, et al. 1990; Silletti, et al. 1991; and Watanabe, et al. 1991).

Many types of host-derived soluble factors act in a paracrine fashion to stimulate cell locomotion.

Motility-stimulating proteins called "scatter factors" have been identified which are produced by embryonic fibroblasts and by smooth muscle cells (Stoker, et al. 1987). Scatter factors stimulate random and directed motility by epithelial cells, keratinocytes, vascular endothelial cells and carcinoma cells (Stoker, et al. 1987; Rosen, et al. 1990; and Weidner, et al. 1990), but not fibroblasts. In addition, a number of host-secreted growth factors have been demonstrated to stimulate motility in tumor cells, including nerve growth factor (Kahan, et al. 1987) insulin-like growth factor-I (Stracke, et al. 1988), interleukin-6 (Tamm, et al. 1989), interleukin-8 (Wang, et al. 1990), and acidic fibroblast growth factor (Jouanneau, et al. 1991). These paracrine factors may influence "homing" or the directionality of tumor cell motility.

In contrast to these host-derived factors, many types of tumor cells have been found to produce proteins termed "autocrine motility factors" which stimulate motility by the same tumor cells which make the factor (Liotta, et al. 1986). Autocrine motility factors are not specific for a given type of cancer cell but have a wide spectrum of activity on many types of cancer cells (Kohn, et al. 1990), with little effect on normal fibroblasts or leukocytes.

Autocrine motility factors identified to date act through cell-surface receptors (Stracke, et al. 1987; Nabi, et al. 1990; Watanabe, et al. 1991) resulting in pseudopodial protrusion (Guirguis, et al. 1987) leading to both random and directed migration (Liotta, et al. 1986; Atnip, et al. 1987; Ohnishi, et al. 1990).

Prior studies of human A2058 melanoma cells have demonstrated that these cells are a particularly rich source of autocrine motility factors. An autocrine motility factor with a molecular mass of approximately 60 kDa has been previously isolated from the conditioned

media of these cells. (Liotta, et al. 1986). Similar tumor cells derived or induced factors with the same molecular weight have subsequently been reported and purified by several investigators (Atnip, et al. 1987; Schnor, et al. 1988; Ohnishi, et al. 1990; Silletti, et al. 1991; Watanabe et al. 1990). Such factors are thought to play a key role in tumor cell invasion.

Most of the motility factors identified to date have not been purified to homogeneity and have not been sequenced. The novel tumor motility factor of the present invention, named herein as autotaxin ("ATX"), has been purified and verified to be a homogeneous sample by two-dimensional gel electrophoresis. The protein of the present invention is unique from any previously identified or purified motility factor. The molecular size of ATX is about 125 kilo Daltons ("kDa") and it has an isoelectric point of approximately 7.7. ATX stimulates both random and directed migration of human A2058 melanoma cells at picomolar concentrations. The activity of the ATX factor is completely sensitive to inhibition by pertussis toxin. No significant homology has been found to exist between the protein of the invention and any mammalian protein including previous factors known to stimulate cell motility.

There is a great clinical need to predict the aggressiveness of a patient's individual tumor, to predict the local recurrence of treated tumors and to identify patients at high risk for development of invasive tumors. The present invention provides a functional marker which is functionally related to the invasive potential of human cancer. The invention further provides an assay for this secreted marker in body fluids, or in tissues. The assay of the invention can be used in the detection, diagnosis, and treatment of human malignancies and other inflammatory, fibrotic, infectious or healing disorders.

SUMMARY OF THE INVENTION

The present invention relates, generally, to a motility stimulating protein and corresponding peptides thereof, and to a DNA segment encoding same. A human cDNA clone encoding a tumor cell motility-stimulating protein, herein referred to as autotaxin or "ATX", reveals that this protein is an ecto/exoenzyme with significant homology to the plasma cell membrane differentiation antigen PC-1. ATX is a 125 kDa glycoprotein, previously isolated from a human melanoma cell line (A2058), which elicits chemotactic and chemokinetic responses at picomolar to nanomolar concentrations.

It is a specific object of the present invention to provide autotaxin and peptide fragments thereof.

It is a further object of the present invention to provide a DNA segment that encodes autotaxin and a recombinant DNA molecule comprising same. It is a further object of the present invention to provide a cell that contains such a recombinant molecule and a method of producing autotaxin using that cell.

Another object of the present invention is the identification of a transmembrane domain of the human liver autotaxin protein and its apparent absence in tumorous forms of autotaxin. The tumorous form of autotaxin appears to be a secreted protein. The present invention relates to utilization of the different sites of localization for diagnosis and prognosis of the stages of tumor progression. Further, the invention relates to treatment methods, designed to advantageously block the secreted form of autotaxin activity while having little effect on the membrane-bound form of autotaxin.

Yet another object of the present invention relates to the identification of a highly variable region within the autotaxin gene, called a "hot spot". The variations in sequence apparently result in mutations, insertions, deletions and premature termination of

translation. The present invention relates to manipulating this region so as to alter the activity of the protein. Further, the hot spot can serve as a marker in tumor diagnosis differentiating between different forms of the autotaxin protein.

It is yet another object of the present invention to provide a method of purifying autotaxin.

It is a further object of the present invention to provide cloned DNA segments encoding autotaxin and fragments thereof. The cDNA encoding the entire autotaxin protein contains 3251 base pairs, and has an mRNA size of approximately 3.3 kb. The full-length deduced amino acid sequence of autotaxin comprises a protein of 915 amino acids. Database analysis of the ATX sequence revealed a 45% amino acid identity (including 30 out of 33 cysteines) with PC-1, a pyrophosphatase/type I phosphodiesterase expressed on the surface of activated B cells and plasma cells. ATX, like PC-1, was found to hydrolyze the type I phosphodiesterase substrate p-nitrophenyl thymidine-5'monophosphate. Autotaxin now defines a novel motility-regulating function for this class of ecto/exo-enzymes.

Further objects and advantages of the present invention will be clear from the description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Fractionation of ATX by hydrophobic interaction. A 200 ml sample of A2058 conditioned media was chromatographed on a 200 mL column of phenyl Sepharose-4B. Buffer A was 50 mM Tris (pH 7.5), 5% methanol, and 1.2 M ammonium sulfate. Buffer B was 50 mM Tris (pH 7.5), 5% methanol and 50% ethylene glycol. The gradient (----) represents a double linear gradient with decreasing ammonium sulfate (1.2 to 0.0 M) and increasing ethylene glycol (0 to 50%). Absorbance was monitored at 280 nm (———) and indicated that most of the protein did not bind to the column. Ten ml fractions were assayed for

motility stimulating capacity using the Boyden Chamber assay (o). The peak of motility activity occurred between 900 and 1050 minutes, ~ 12% of the gradient.

Figure 2. Isolation of ATX by lectin affinity chromatography. 20 ml portions of the phenyl Sepharose activity peak were affinity purified on a 40 ml Concanavalin A Affi-Gel column. The bound components were eluted with a step gradient (----) of methyl α-D-mannopyranoside (0.0 mM, 10 mM, and 500 mM) in a buffer consisting of 0.05 M Tris (pH 7.5), 0.1 M NaCl, 0.01 M $CaCl_2$ and 20% ethylene glycol. Absorbance was monitored at 280 nm (_____) and indicated that the majority of the protein components did not bind to the column. Motility was assayed in 10 mL fractions (...o...) and was found predominantly in the 500 mM elution concentration. One of seven chromatographic runs is shown.

Figure 3. Purification of ATX by weak anionic exchange chromatography. Approximately 30% of the activity peak eluted from the Con A affinity column was applied to a ZORBAX BioSeries-WAX column. The bound components were eluted with an NaCl gradient (----) in a buffer consisting of 10 mM Tris (pH 7.5) and 30% ethylene glycol. Motility (o) was assayed in 1.0 ml fractions. The peak of activity eluted in a discrete but broad region in the shallow portion of the gradient. Absorbance was monitored at 230 nm (_____). The majority of the protein components not associated with activity bound strongly to the column were eluted at 1.0 M NaCl. One of two chromatographic runs is shown.

Figure 4. Purification of ATX by molecular sieve exclusion chromatography. The entire activity peak eluted from the weak anion exchange column was applied to a series of TSK columns (4000SW, 4000SW, 3000SW, and 2000SW, in this order). Proteins were eluted in a buffer consisting of 0.1M $NaPO_4$ (pH 7.2) with 10% methanol and 10% ethylene glycol. Two major protein peaks were evident

by monitoring the absorbance at 235 nm (_____). Motility (...o...) was assayed in 0.4 ml samples and found predominantly in the first, smaller, protein peak.

Figure 5. Final purification of ATX by strong anionic exchange chromatography. Approximately 15% of the activity peak from the molecular sieve exclusion series was applied to a Pro-Pac PA1 column. Protein which bound to the column was eluted with a NaCl gradient (----) in a buffer consisting of 10 mM Tris (pH 7.5), 5% methanol and 20% ethylene glycol. Absorbance was monitored at 215 nM (_____). Motility activity was assayed in 1.0 ml fractions at two different dilutions: 1/5 (...o....) or 1/15 (.__.o.__.). Activity was found to correspond to a double protein peak in the central region of the gradient.

Figure 6A, 6B and 6C. Protein components associated with the activity peaks from various stages of purification. The activity peak from each chromatographic fractionation was pooled, concentrated and analyzed by SDS-polyacrylamide gel electrophoresis. Molecular weight standards are in Lane 1 for each panel. Panel 6A) 8-16% gradient gel of the first three purification steps, run under non-reducing conditions. Lane 2 is an aliquot of the pooled activity peak eluted from the phenyl sepharose fractionation. Lane 3 is an aliquot of the pooled activity peak eluted from the Con A affinity purification. Lanes 4 and 5 show the "peak" and "shoulder" of activity fractionated by weak anion exchange chromatography (Figure 3). Panel 6B) 7% gel of the activity peak fractionated by molecular sieve exclusion chromatography. Lanes 2 and 3 show the protein separation pattern of the total pooled activity peak when the gel was run under non-reducing and reducing conditions, respectively. Panel 6C) 8-16% gradient gel of the final strong anionic exchange chromatographic separation, run under non-reducing conditions. Lane 2 comprises ~1% of the total pooled activity peak eluted from the column.

Figure 7. Two-dimensional gel electrophoresis of ATX. Purified ATX (Figure 6, Panel C) was subjected to non-equilibrium isoelectric focusing (5 hr. at 500v), then applied to a 7.5% SDS-polyacrylamide gel for the second dimension. The pH separation which resulted was measured in 0.5 cm samples of concurrently run tube gels and is shown at the top. Molecular weight standards for the second dimension are shown on the right. This analysis reveals a single component with pI = 7.7 ± 0.2 and M_r = 120,000.

Figure 8. Dilution curve of ATX. Purified ATX (Figure 6, Panel C) was serially diluted and tested for motility-stimulating activity. The result, with unstimulated background motility subtracted out, shows that activity is half-maximal at ~ 500 pM ATX.

Figure 9. Pertussis toxin (PT) sensitivity of ATX. A2058 cells were pre-treated for 1 hr. prior to the start of the motility assay with 0.5 μg/ml PT in 0.1% BSA-DMEM or with 0.1% BSA-DMEM alone (for untreated control). The motility activity stimulated by purified ATX (Figure 6, Panel C) was then assessed for the two treatment groups. The result, expressed as cells/HPF ± S.E.M. with unstimulated background motility subtracted out, reveals profound inhibition of PT-treated cells (hatched) compared to untreated cells (solid). PT had no effect on cell viability. S.E.M.'s were < 10%.

Figure 10. Checkerboard analysis of ATX-stimulated motility. Varying dilutions of autotaxin were added to the upper chamber with the cells and/or to the lower chamber, as shown. Motility response, expressed as cells/HPF ± S.E.M., was assessed for each point in the checkerboard.

Figure 11. Purification of ATX peptides on HPLC. ATX, purified to homogeneity by strong anionic exchange chromatography, was sequentially digested by cyanogen bromide, subjected to reduction and

pyridylethylation, and digested by trypsin. The resulting peptides were purified on an Aquapore RP300 C-8 reverse phase column using a (0-70)% acetonitrile gradient in 0.1% trifluoroacetic acid (----). The absorbance was monitored at 215 nm (_____) and peaks were collected. Seven peaks, chosen at random for N-terminal amino acid sequence analysis, are shown with appropriate numbers.

Figure 12. Cloning Strategy, schematically depicted.

Figure 13. Schematic Diagram of autotaxin gene region.

For A2058: 4C11 is the original DNA clone obtained by screening an A2058 cDNA expression library in λgt11 with anti-peptide ATX-102. Upstream ATX peptide sequences were utilized for PCR amplification of A2058 mRNA, using the technique of reverse transcription/PRC. These peptides include ATX-101, ATX-103, and ATX-224. The approximate localization of each of peptide was obtained by matching the peptide with its homologous region on PC-1.

For N-tera 2D1, a λgt1 0 cDNA library was amplified and the cDNA inserts were isolated. PCR amplification, based on homologies with A2058 sequence, was utilized for DNA sequencing.

For normal human liver, a mRNA from liver was amplified with 5'RACE using primers from the known ATX-224 region of A2058 and N-tera 2D1.

Figure 14. Schematic match-up of ATX peptides with putative A2058 protein sequence.

Figure 15. Schematic match-up of ATX peptides with putative N-tera 2D1 protein sequence.

Figure 16: ATX Treatment with PGNase F. Partially purified ATX was treated with 60 mU/ml PNGase F at 37°C for 16 hr under increasingly denaturing conditions. The treated ATX samples were separated by SDS polyacrylamide gel electrophoresis run under reducing

conditions and stained with Coomassie blue G-250. Lane 1 contains untreated ATX (arrow) with no enzyme added. Lane 2 contains the reaction mixture run under non-denaturing conditions (50 mM tris/10% ethylene glycol, pH 7). Lanes 3 and 4 have added 0.1 M β-mercaptoethanol and 0.5% Nonidet-P40, respectively. Lanes 5 and 6 contain the reaction mixtures in which the ATX sample was first boiled for 3 min in 0.1% SDS with (lane 6) or without (lane 5) 0.1 M β-mercaptoethanol, then had 0.5% Nonidet-P40 added to prevent enzyme denaturation. The enzyme can be detected as an ~44 kDa band in lanes 2-6.

Figure 17: Effect of varying concentrations of PNGase F on ATX molecular weight and motility-stimulating activity. Partially purified ATX was treated with various concentrations (range 0 - 60 mU/ml, shown on horizontal axis) of PNGase F at 37°C for 16 hr. Figure 17A shows the effect of the different treatments on ATX molecular weight. At concentrations of enzyme $\geq$ 30 mU/ml, the deglycosylation reaction appears to be complete. Figure 17B shows the effect of the identical reaction mixtures on motility-stimulating capacity (immediately below the corresponding protein band of Figure 17A). There is no significant difference between any of the treatment groups.

Figure 18: Comparison of amino acid sequences of ATX and PC-1. The amino acid sequences of ATX and PC-1 are compared. Amino acid identity is indicated by a vertical line between the sequences. The location of the putative transmembrane/signal sequence is shown by a solid line. The two somatomedin B domains are identified by dashed lines. The putative phosphodiesterase active site is indicated by emboldened lines. The loop region of a single EF hand loop region is identified with double lines. The presumed cleavage site for each protein is indicated with arrows.

Figure 19: Domain structure of ATX and PC-1.

Putative domains are indicated for the two homologous proteins, ATX and PC-1.

DETAILED DESCRIPTION OF THE INVENTION

Tumor cell motility is a critical component of invasion and metatasis, but the regulation of this motility is still poorly understood. At least some tumor cells secrete autocrine motility factors (AMF's) that stimulate motility in the producing cells. Like the analogous autocrine growth factors, these AMF's allow tumor cells independence from the host in this important component of the metastatic cascade. One AMF, autotaxin (ATX), has recently been purified to homogeneity from the human melanoma cell line, A2058 (Stracke, et al., 1992). The purified protein was enzymatically digested and the peptide fragments were separated by reverse phase HPLC. A number of these peptides have been sequenced by standard Edman degradation (Table 6) from different purifications and different enzymatic digestion. Sequence information, obtained initially on 19 purified tryptic peptides, confirmed that the protein is unique with no significant homology to growth factors or previously described motility factors. These peptide sequences have now been used as the basis for identifying and sequencing the cDNA clone for ATX. The present invention comprises an amino acid sequence of ATX as well as a nucleic acid sequence coding for the ATX protein.

TABLE 6. PEPTIDE SEQUENCES FOR AUTOTAXIN.

PEPTIDE NO.	AMINO ACID SEQUENCE	SEQ ID: NO:
ATX-18	WHVAR	SEQ ID NO:1
ATX-19	PLDVYK	SEQ ID NO:2
ATX-20	YPAFK	SEQ ID NO:3
ATX-29	PEEVTRPNYL	SEQ ID NO:5

ATX-34B	RVWNYFQR	SEQ ID NO:38
ATX-41	HLLYGRPAVLY	SEQ ID NO:29
ATX-48	VPPFENIELY	SEQ ID NO:7
ATX-59	TFPNLYTFATGLY	SEQ ID NO:32
ATX-100	GGQPLWITATK	SEQ ID NO:8
ATX-101/223A	VNSMQTVFVGYGPTFK	SEQ ID NO:9
ATX-102	DIEHLTSLDFFR	SEQ ID NO:10
ATX-103	TEFLSNYLTNVDDITLVPETLGR	SEQ ID NO:11
ATX-104	VNVISGPIDDYDYDGLHDTEDK	SEQ ID NO:33
ATX-204	MHTARVRD	SEQ ID NO:39
ATX-205	FSNNAKYD	SEQ ID NO:40
ATX-209	VMPNIEK	SEQ ID NO:41
ATX-210	TARGWECT	SEQ ID NO:42
ATX-212	(N)DSPWT(N)ISGS	SEQ ID NO:43
ATX-214	LRSCGTHSPYM	SEQ ID NO:44
ATX-215/34A	TYLHTYES	SEQ ID NO:45
ATX-213/217A	AIIANLTCKKPDQ	SEQ ID NO:46
ATX-216	IVGQLMDG	SEQ ID NO:47
ATX-218/44	TSRSYPEIL	SEQ ID NO:48
ATX-223B/24	QAEVSSVPD	SEQ ID NO:49
ATX-224	RCFELQEAGPPDDC	SEQ ID NO:50
ATX-229	SYTSCCHDFDEL	SEQ ID NO:51
ATX-244/53	QMSYGFLFPPYLSSSP	SEQ ID NO:52

ATX is a glycosylated protein due to its high affinity for concanavalin A and amino acid sequence analysis of the ATX peptides. ATX has been demonstrated to be a 125kDa glycoprotein whose molecular weight reduced to 100-105kDa after deglycosylation with N-glycosidase F. The calculated molecular weight of the cloned protein is 100kDa (secreted form) or 105kDa (full length protein). Based on amino acid composition, the estimated pI is 9.0 which is higher than the pI determined by 2-D gel

electrophoresis analysis (7.7-8.0) of purified ATX. This difference can be explained by the presence of sialic acid residues on the sugar moieties.

Autotaxin is secreted by A2058 human melanoma cells cultured in low abundance in serum-free conditioned medium. Autotaxin is a potent new cytokine with molecular mass 125 kDa which has been purified to homogeneity from the conditioned medium of the human melanoma cell line, A2058, utilizing sequential chromatographic methods as described herein. This new cytokine, termed autotaxin (ATX), is a basic glycoprotein with pI ~ 7.8. ATX is active in the high picomolar to low nanomolar range, stimulating both chemotactic and chemokinetic responses in the ATX-producing A2058 cells as well as other tumor cells. This motile response is abolished by pretreatment of the cells with pertussis toxin. ATX may therefore act through a G protein-linked cell surface receptor. These characteristics distinguish ATX from several small growth factors and interleukins which are implicated in tumor cell motility (Stracke et al., 1988; Ruff et al., 1985; Maciag et al., 1984; Gospodarowicz, 1984; Van Snick, 1990; Yoshimura 1987).

The protein of the present invention, which in one embodiment is derived from A2058 human melanoma cells, can be prepared substantially free from proteins with which it is normally associated using, for example, the purification protocol disclosed herein. Alternatively, the protein of the present invention can be prepared substantially free from proteins, by cloning and expressing the cDNA encoding autotaxin as disclosed herein.

A large volume of serum-free conditioned medium from appropriate producer cells (e.g., tumor cells) is collected and concentrated approximately 500-fold. This concentrated conditioned medium is then separated from other contaminating proteins by techniques that rely on

the chemical and physical characteristics of the protein. These include the molecular weight, relative hydrophobicity, net charge, isoelectric focusing point, and the presence of lectin-binding sugar residues on the protein.

Alternatively, the protein, or functional portion thereof, can be synthesized using chemical or recombinant means.

The protein of the present invention has a potent biological activity. Purified ATX is active in the picomolar range and 1 unit of activity corresponds to a concentration of approximately 500 pM as assessed by the cell motility assay described herein and elsewhere (Stracke et al., 1989).

The protein of the present invention has a molecular size, as determined by two dimensional gel electrophoresis, of from 120 to 130 kDa, or more specifically, about 125 kDa. Further, the protein of the present invention can have a pI in the range of 7.5 to 8.0, preferably, approximately 7.7. The present invention relates to autotaxin and peptides thereof having cell motility properties as described herein. These proteins and peptides thereof can be produced by isolation from a natural host or isolation as an expression product from a recombinant host.

The present invention also relates to a DNA segment coding for a polypeptide comprising an amino acid sequence corresponding to ATX, or a unique portion of such a sequence (unique portion being defined herein as at least 5, 10, 25, or 50 amino acids). In one embodiment, the DNA segment encodes any one of the amino acid sequences shown in SEQ ID NO:1 to SEQ ID NO:11 and SEQ ID NO:26 to SEQ ID NO:33. Another embodiment uses larger DNA fragments encoding amino acid sequences shown in SEQ ID NO:34, SEQ ID NO: 36 and SEQ ID NO:38. The entire coding region for autotaxin can also be used in the present invention shown

in SEQ ID NO:66 through SEQ ID NO:69.

In another embodiment, the present invention relates to a recombinant DNA molecule comprising a vector (for example plasmid or viral vector) and a DNA segment coding for a polypeptide corresponding to ATX, as can be prepared by one skilled in the art. Preferably, the coding segment is present in the vector operably linked to a promoter. The present invention also relates to a recombinant protein produced from a host cell expressing a cDNA containing a coding region of ATX. Examples of ATX cDNAs from a variety of sources have been cloned and can be used for expression, including *inter alia* A2058 carcinoma cells, N-tera 2D1 cells and human liver.

In a further embodiment, the present invention relates to a cell containing the above-described recombinant DNA molecule. Suitable host cells include procaryotic cells (such as bacteria, including E. coli) and both lower eucaryotic cells (for example, yeast) and higher eucaryotic cells (for example, mammalian cells). Introduction of the recombinant molecule into the host cells can be effected using methods known in the art.

In another embodiment, the present invention relates to a method of producing a polypeptide having an amino acid sequence corresponding to ATX. The method comprises culturing the above-described cells under conditions such that the DNA segment is expressed, and isolating ATX thereby produced.

In a further embodiment, the present invention relates to an antibody having affinity for ATX or peptide fragments thereof. The invention also relates to binding fragments of such antibodies. In one preferred embodiment, the antibodies are specific for ATX peptides having an amino acid sequence set forth in one of SEQ ID NO:1 through SEQ ID NO:11 and SEQ ID NO:26 through SEQ ID NO:34, SEQ ID NO: 36 and SEQ ID NO:38 through SEQ ID NO:52. In addition, the antibodies may recognize an

entire autotaxin protein.

Antibodies can be raised to autotaxin or its fragment peptides, either naturally-occurring or recombinantly produced, using methods known in the art.

The ATX protein and peptide fragments thereof described above can be joined to other materials, particularly polypeptides, as fused or covalently joined polypeptides to be used as carrier proteins. In particular, ATX fragments can be fused or covalently linked to a variety of carrier proteins, such as keyhole limpet hemocyanin, bovine serum albumin, tetanus toxoid, etc. See for example, Harper and Row, (1969); Landsteiner, (1962); and Williams et al., (1967), for descriptions of methods of preparing polyclonal antisera. A typical method involves hyperimmunization of an animal with an antigen. The blood of the animal is then collected shortly after the repeated immunizations and the gamma globulin is isolated.

In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts. Description of techniques for preparing such monoclonal antibodies may be found in Stites et al., and references cited therein, and in particular in Kohler and Milstein (1975), which discusses one method of generating monoclonal antibodies.

In another embodiment, the present invention relates to an oligonucleotide probe synthesized according to the sense or antisense degenerative sequence set forth in one of SEQ ID NO:1 through SEQ ID NO:11, SEQ ID NO:26 through SEQ ID NO:33, SEQ ID NO:39 through SEQ ID NO:52, and SEQ ID NO:55 through SEQ ID NO:65.

Protein database searches of this sequence revealed a 45% amino acid identity with the plasma cell membrane marker protein, PC-1. ATX and PC-1 appear to share a number of domains, including two somatomedin B domains, the loop region of an EF hand, and the enzymatic site of

type I phosphodiesterase/ nucleotide pyrophosphatase. Like PC-1, ATX hydrolyzes p-nitrophenyl thymidine-5' - monophosphate, a type 1 phosphodiesterase substrate. This enzymatic function of ATX suggests a newly identified function for ecto/exo-enzymes in cellular motility.

In a further embodiment, the present invention relates to a method of diagnosing cancer metastasis and to a kit suitable for use in such a method. Preferably, antibodies to ATX can be used in, but not limited to, ELISA, RIA or immunoblots configurations to detect the presence of ATX in body fluids of patients (e.g. serum, urine, pleural effusions, etc.). These antibodies can also be used in immunostains of patient samples to detect the presence of ATX.

In yet another embodiment, the present invention relates to *in vivo* and *in vitro* diagnostics. ATX may be radiolabelled, by means known to one skilled in the art, and injected in cancer patients with appropriate ancillary substances also known to one skilled in the art, in order to ultimately detect distant metastatic sites by appropriate imagery. The level of ATX in tissue or body fluids can be used to predict disease outcomes and/or choice of therapy which may also include ATX inhibitors.

In a further embodiment, the present invention relates to a treatment of cancer. ATX antibodies can be cross-linked to toxins (e.g., Ricin A), by means known to one skilled in the art, wherein the cross-linked complex is administered to cancer patients with appropriate ancillary agents by means known to one skilled in the art, so that when the antibody complex binds to the cancer cell, the cell is killed by the cross-linked toxin.

In another embodiment, the different localizations of the normal versus tumorous forms of the ATX proteins within the tissue can be used as a tool for diagnosis and prognosis. The stage of disease progression can be monitored by elevated levels of ATX in the extracellular

space as opposed to its normal cell membranes association. In addition, treatment methods for control of tumor progression can be designed to specifically block the activity of the secreted form of ATX. Such methods would have a preferential effect upon secreted ATX during tumor progression while not effecting normal ATX formation.

Yet another embodiment utilizes the hot spot located in the region from approximately nucleotides 1670 through 1815, as a marker gene for identification of tissues carrying a tumorous form of ATX.

The present invention is described in further detail in the following non-limiting examples.

EXAMPLES

The following protocols and experimental details are referenced in the Examples that follow:

Materials. The polycarbonate Nuclepore membranes and the 48-well microchemotaxis chambers were obtained from Neuro Probe, Inc. Pertussis toxin (PT), ethylene glycol (biotechnology grade), methyl α-D-mannopyranoside were obtained from commercial vendors. The ampholyte, pH 3-10 Bio-Lyte and pH 8-10 Bio-Lyte, were obtained from Bio-Rad. Phenyl Sepharose CL-4B; affi-Gel concanavalin A; ZORBAX BioSeries-WAX (weak anion exchange) column (9.4mm x 24cm); Spherogel-TSK 4000SW, 3000SW and 2000SW columns (each 7.5mm x 30cm); the Pro-Pac PA1 (4 x 50mm) strong anion exchange column; the Aquapore RP300 C-8 reverse phase column (220 x 2.1mm); and the AminoQuant C-18 reverse phase column (200 x 2.1mm) were also obtained from commercial sources.

Affi-Gel 10 affinity resin was from Bio-Rad. The GeneAmp PCR Reagent kit with AmpliTaq and the GeneAmp RNA PCR kit were purchased from Perkin-Elmer. The 5' RACE kit came from Gibco BRL Life Technologies, Inc. The p-nitrophenyl thymidine-5'monophosphate was obtained from Calbiochem Biochemicals.

Ethylene glycol (biotechnology grade) was from Fisher

Biochemicals (Pittsburg, PA). Peptide N-glycosidase F ("PNGase F"), O-glycosidase, neuraminidase (*Arthrobacter ureafaciens*), and swainsonine ("Swn") came from Boehringer-Mannheim (Indianapolis, IN). 1-Deoxymannojirimycin ("dMAN"), and N-methyl-1-deoxynojirimycin ("NMdNM") were from Oxford GlycoSystems, Inc. (Rosedale, NY). Biotinylated concanavalin A, HRP-conjugated streptavidin, and HRP-conjugated goat anti-rabbit immunoglobulin were purchased from Pierce Chemicals (Rockford, IL). Polyvinyl pyrrolidone-free polycarbonate membranes and the microchemotaxis chamber were from NeuroProbe, Inc. (Cabin John, MD).

Cell Culture. The human melanoma cell line A2058, originally isolated by Todaro (Todaro et al., 1980), was maintained as previously described by Liotta (Liotta et al., 1986). The N-tera 2 (D1 clone) was a kind gift from Dr. Maxine Singer, Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health and was maintained as described (Andrews, P.W., Goodfellow, P.N. and Bronson, D.L. (1983) *Cell surface characteristics and other markers of differentiation of human teratocarcinoma cells in culture*.).

Production of Autotaxin. A2058 cells were grown up in T-150 flasks, trypsinized, and seeded into 24,000 cm^2 cell factories at a cell density of $1x10^{10}$ cells/factory. After 5-6 days, the serum-containing medium was removed and the cells were washed with DPBS. The factories were maintained in DMEM without phenol red, supplemented with 4 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 5 μg/ml crystallized bovine serum albumin, 10 μg/ml bovine insulin, and 1 μM aprotinin. Culture supernatants were harvested every 3 days, frozen at -40°C and replaced with fresh serum-free medium. Each cycle of supernatant was tested for ATX production with a cell motility assay detailed below. Typically, a cell factory continued to be productive for 9-11 of these cycles.

After accumulation of approximately 45-60 L of supernatant, the culture supernatants were thawed and concentrated down to 2-2.5 L using an Amicon S10Y30 spiral membrane ultrafiltration cartridge. This supernatant was further concentrated in an Amicon high performance ultrafiltration cell using Diaflo membranes. The final volume achieved from 100-200 L of conditioned medium was typically 250-400 ml. All ultrafiltrations were performed at 4°C.

Cell Motility Assays. Purification of autotaxin was monitored by testing the motility-stimulating capacity of the fractions collected from the columns. These fractions were in buffers unsuitable for a chemotaxis assay so each fraction had to be washed into an appropriate buffer, i.e., 0.1% (w/v) BSA in DPBS containing calcium and magnesium. This dialysis was performed by adding aliquots of each fraction to be tested into Centricon-30™ ultrafiltration tubes, which retain molecular species larger than 30,000 daltons.

The assay to determine motility was performed in triplicate using a 48-well microchemotaxis chamber as described elsewhere in detail (Stracke et al., 1987; Stracke, et al., 1989). The Nuclepore™ membranes used in these modified Boyden chambers were fixed and stained with Diff-Quik.™ Chemotaxis was quantitated either by reading the stained membranes with a 2202 Ultroscan laser densitometer or by counting 5 randomly chosen high power fields (HPF) under light microscopy (400 x) for each replicate. Densitometer units (wavelength - 633 nm) have been shown to be linearly related to the number of cells per HPF (Taraboletti, 1987; Stracke, et al., 1989). Typically, unstimulated motility (background) corresponded to 5-10 cells/HPF and highly responding cells to 70-100 cells/HPF above unstimulated background (i.e., 75-110 total cells/HPF).

For experiments using PT, the toxin was pre-incubated

3 ml/min.

The active peak was pooled, dialyzed against 0.1 M sodium phosphate (pH 7.2), 10% (v/v) methanol, and 10% (v/v) ethylene glycol, and subjected to a fourth fractionation step on a series of Spherigel TSK columns (4000SW, 4000SW, 3000SW, 2000SW, in that order). This molecular sieve step was run using the Shimadzu BioLiquid chromatograph at 0.4 ml/min.

The active peak was pooled and dialyzed into 10 mM Tris (pH 7.5), 5% (v/v) methanol, 20% (v/v) ethylene glycol and subjected to a fifth (strong anion exchange) chromatography step, a Pro-Pac PA1 column run at 1 ml/min using a Dionex BioLC with AI450 software. The sample was eluted with a linear gradient of (0.0-0.4M) NaCl.

In order to calculate activity yields after each step of purification, a unit of activity had to be derived. The dilution curve of ATX was biphasic with a broad peak and a linear range at sub-optimal concentrations. One unit of activity/well (i.e., 40 units/ml) was defined as 50% of the maximal activity in a full dilution curve. This allowed calculation of the activity contained in any volume from the dilution needed to achieve 1 unit/well. Therefore, if a 1:10 dilution were needed in order to produce 1 unit of activity/well, the material contained 10 x 40 = 400 units/ml.

Gel Electrophoresis. Protein samples were analyzed by SDS-polyacrylamide gel electrophoresis using the conditions of Laemmli (Laemmli, 1970). In brief, 7 or 8% SDS-containing polyacrylamide gels were prepared or pre-poured (8-16%) gradient gels were obtained commercially. Samples were prepared with or without reducing conditions (5% β-mercaptoethanol). After electrophoretic separation, the gels were stained using Coomassie Blue G-250 as previously described (Neuhoff, et al., 1988). In this staining protocol, which ordinarily requires no destaining step, the Coomassie stain appears to be able to stain as little

as 10 ng of protein.

For two-dimensional electrophoresis, the protein, in 20% ethylene glycol, was dried in a Speed-vac and redissolved in loading solution: 9M urea, 1% (v/v) pH 3-10 Bio-Lyte, and 2.5% (v/v) Nonidet-P40. This sample was then subjected to isoelectric focusing (O'Farrell, 1975) using a Bio-Rad tube cell in 120 x 3 mm polyacrylamide tube gels containing 9M urea, 2% (v/v) pH 3-10 Bio-Lyte, 0.25% (v/v) pH 8-10 Bio-Lyte and 2.5% (v/v) Nonidet-P40. Reservoir solutions were 0.01 M phosphoric acid and 0.02 M NaOH. Non-equilibrium isoelectric focusing (O'Farrell, et al., 1977) was run initially with constant voltage (500 v) for 5 hr. Since the protein was basic, the procedure was repeated under equilibrium conditions (500 v for 17 hr.). Electrophoresis in the second dimension was performed on a 7.5% polyacrylamide using the conditions of Laemmli (1970). The gel was stained with Coomassie Blue G-250 as above.

Preparation of peptides for internal sequence of autotaxin. Homogeneous ATX was sequentially digested with cyanogen bromide and, following reduction and pyridylethylation, with trypsin (Stone, et al., 1989). The resulting fragments were then separated by gradient elution on an Aquapore RP300 C-8 reverse phase column: 0.1% (v/v) trifluoroacetic acid and (0-70)% acetonitrile over 85 min. at a flow rate of 0.2 ml/min. A Dionex AI450 BioLC system was utilized and fractions were collected manually while monitoring the absorbance at 215 nm.

Sequence analysis of peptides. The amino acid sequences of peptides resulting from digestion and purification of ATX peptides #1-7 and 12-18, corresponding to SEQ ID NO:1 through SEQ ID NO:7 and SEQ ID NO:26 through SEQ ID NO:32, respectively, were determined on a Porton Instruments 2020 off-line sequenator using standard program #1. Phenylthiohydantoin amino acid analysis of sequenator runs were performed on a Beckman System Gold HPLC using a

modified sodium acetate gradient program and a Hewlett-Packard C-18 column. ATX-100 (SEQ ID NO:8), ATX-101 (SEQ ID NO:9), ATX-102 (SEQ ID NO:10), ATX-103 (SEQ ID NO:11) and ATX 104 (SEQ ID NO:33) were sequenced from gel-purified ATX.

Protein databases (Pearson, et al. 1988) that were searched for homologies in amino acid sequence with the ATX peptides include: GenBank (68.0), EMBL (27.0), SWISS-PROT (18.0), and GenPept (64.3).

EXAMPLE 1

Purification of Autotaxin

The A2058 cells had been previously shown to produce protein factors which stimulate motility in an autocrine fashion (Liotta, et al., 1986). Conditioned medium from these cells was therefore used to identify and purify a new motility-stimulating factor, which is here named autotaxin and referred to as ATX. Since the purification was monitored with a biological assay, motility-stimulating activity had to be maintained throughout. The activity proved to be labile to freezing, acidic buffers, proteases (but not DNase or RNase), reduction, strong chaotrophic agents (e.g. > 4 M urea), and a variety of organic solvents (isopropanol, ethanol, acetonitrile). An organic solvent, ethylene glycol, which did not decrease bioactivity, was added for both storage and chromatographic separation.

100-200 L of serum-free conditioned medium were required in order to produce enough ATX for amino acid sequence analysis. The medium contained low concentrations of both BSA (5 μg/ml) which was needed as a carrier protein and insulin (10 μg/ml) which was required to support cell growth in low protein medium. Ultrafiltration to concentrate this large volume was performed with low protein-binding YM30 membranes which retain molecular species with M_r > 30,000. As seen in Table 1, 200 L of conditioned medium prepared in this

manner resulted in 10 x 10^6 units of activity. However, the initial unfractionated conditioned medium contained additional substances known to stimulate activity, particularly insulin, which does not completely wash out in the ultrafiltration step and which is additive to the motility stimulating activity in a complex manner (Stracke, et al., 1989). This had to be taken into account in order to determine yields for subsequent steps in which insulin had been removed.

TABLE 1. PURIFICATION OF AUTOTAXIN

Purification Step	Protein (mg)	Activity [a] (total units)	Specific Activity (units/mg)	Recovery (%)[b]
200 L Conditioned Medium	33,000	10,000,000[c]	300	
Phenyl Sepharose	1,235	460,000	370	100
Concanavalin A	58	660,000	11,400	100
Weak Anion Exchange	4.5	490,000	110,000	100
TSK Molecular Sieves	~0.4[d]	220,000	550,000	48
Strong Anion Exchange	~0.04[d]	24,000[e]	600,000	5.2

[a] Activity calculated from Boyden chamber assay. The dilution which resulted in 50% of maximal activity (generally approximately 20 laser density units or ~40 cells/HPF) was chosen to have 1 unit of activity per well (equivalent to 40 units/ml).

[b] Recovery was estimated from activity, after the first purification column (i.e., phenyl sepharose).

[c] Initial activity in the unfractionated conditioned medium reflected the fact that insulin was used in the medium as a necessary growth factor under low protein conditions.

[d] Estimated protein is based on quantification by amino acid analysis.

[e] This specific activity for purified protein corresponds to ~ 10 fmol ATX/unit of motility activity (in a Boyden chamber well).

The first step in the purification involved fractionation by hydrophobic interaction chromatography using a phenyl Sepharose CL-4B column. The results are shown in Figure 1. Most proteins, including insulin, eluted from the column in early fractions or in the void. However, the peak of activity eluted relatively late. The activity which was purified was estimated as 460,000 units ± 20% (Table 1). As the pooled peak of activity from the phenyl Sepharose fractionation is considered to be the first sample without significant insulin contamination, subsequent yields are measured against its total activity. Gel electrophoresis of a small portion of the pooled peak of activity (Figure 6A, column 2) revealed a large number of protein bands with BSA predominant from the original conditioned medium.

In the second step of purification, the active peak was applied to the lectin affinity column, Affi-Gel concanavalin A. As shown in Figure 2, most protein (estimated to be 90% of the total absorbance at 280 nm) failed to bind to the column at all. The non-binding fraction contained essentially no motility-stimulating activity (see dotted line in Figure 2). When a linear gradient of methyl α-D-mannopyranoside was applied to the column, chemotactic activity eluted off in a prolonged zone, beginning at a concentration of approximately 20 mM sugar. Consequently, a step gradient was used to elute. Pure BSA failed to bind to con A.

Activity was found primarily in the 500 mM step of methyl α-D-mannopyranoside. There appeared to be no significant loss of activity as seen in Table 1; however, specific activity (activity/mg total protein) increased thirty-fold. Gel electrophoresis of the pooled and

concentrated peak (Figure 6A, column 3) revealed that the BSA overload was no longer apparent and the number of bands were much reduced. When the unbound protein was concentrated and applied to a gel, it appeared identical to the active peak from phenyl Sepharose-4B with a large BSA band.

The third purification step involved fractionating the previous active peak by weak anion exchange chromatography as shown in Figure 3. Under the running conditions, activity eluted in a broad peak-shoulder or double peak in the middle of the shallow portion (0.0-0.4 M) of the NaCl gradient. The largest proportion of protein, lacking in motility-stimulating capacity, bound strongly to the column and eluted off in high salt (1 M NaCl). There appeared to be no significant loss of activity, though specific activity increased by twenty-fold (Table 1). Analysis by gel electrophoresis of both the peak (28-34 min. in Figure 3) and the shoulder (35-45 min. in Figure 3) is shown in Figure 6A (columns 4 and 5, respectively). Two predominant protein bands resulted: a broad doublet around 25-35 kDa and a second doublet around 110-130 kDa.

In the fourth purification step, the active peak was applied to a series of molecular sieves. Spectrophotometric monitoring of the eluant revealed two large peaks of protein (Figure 4). Activity corresponded to the first, higher molecular weight peak. Recovery of activity was ~48% with a five-fold increase in specific activity. Analysis by gel electrophoresis was performed under non-reducing and reducing conditions as shown in Figure 6B (columns 2 and 3, respectively). This fractionation step had essentially removed all contaminating protein of molecular weight < 55 kDa. The predominant band remaining has a molecular weight of 120 kDa unreduced and 125 kDa reduced; there are two minor bands with molecular weights 85 kDa and 60 kDa. The fact

that the 120 kDa protein changes so little in electrophoretic mobility after reduction would tend to indicate a paucity of disulfide bonds. However, the existing disulfide bonds have functional significance because motility-stimulating activity is labile to reduction.

The fifth purification step involved fractionation of the active peak by strong anion exchange chromatography. As shown in Figure 5, activity corresponds to two broad optical absorbance peaks in the middle of the gradient with contaminating proteins eluting earlier. These two peaks were identical by amino acid analysis and by polyacrylamide gel electrophoretic separation. They presumably represent different glycosylation states of the same parent protein. Activity is shown in Figure 5 at two different sample dilutions. Several dilutions of the fractionated samples were often necessary in order to resolve the true "peak" of activity as the shape of the ATX dilution curve was not sharp due to saturation and down regulation at high concentrations. Recovery from this chromatographic step is lower (5% compared to phenyl Sepharose), as might be expected when a minute quantity of protein is applied to a column; however, specific activity again increased (Table 1). Analysis by gel electrophoresis revealed a single protein band at molecular weight 120 kDa, unreduced (Figure 6C, column 2).

EXAMPLE 2

Characterization of Autotaxin

Two dimensional gel electrophoresis of the purified protein (Figure 7) revealed a single predominant band. The band slopes downward slightly toward the basic side of the gel in a manner that is characteristic of glycosylated proteins. A basic pI of 7.7 ± 0.2 was essentially the same whether the isoelectric focusing was run under non-equilibrium conditions (5 hr.) or was allowed to go to equilibrium (17 hr.).

A dilution curve of the purified protein is shown in Figure 8. The protein is active in the picomolar range and 1 unit of activity appears to correspond to a concentration of 400-600 picomolar (or approximately 10 fmol of ATX/Boyden chamber well). When dilutions were begun at higher concentrations of ATX, the resultant curve showed a broad plateau with down-regulation at the highest concentrations. The motility response to purified autotaxin is highly sensitive to pertussis toxin (hereinafter referred to as "PT") (Table 2 and Figure 9) with approximately 95% inhibition of activity at 0.5 μg/ml PT.

TABLE 2. Effect of Pertussis Toxin (PT) on Autotaxin-stimulated motility

	A2058 Motility Response (density units[1])	
	control cells[2]	Pertussis toxin-treated cells[3]
Condition medium[4]	60.3	0.4
Purified Autotaxin	38.5	0.0

[1] Chemotaxis quantitated by motility assay (Stracke, et al., 1978).

[2] A2058 cell suspended at 2 x 10^6 cells/ml in DMEM supplemented with 1 mg/ml bovine serum and rocked at room temperature for 1 hr.

[3] As control with 0.5 μg/ml pertussis toxin.

[4] Prepared by adding DMEM without phenol red supplemented with 0.1 mg/ml bovine serum albumin to subconfluent flasks of A2058 cells. The medium was harvested after 2 days incubation at 37°C in a humidified atmosphere and concentrated 25-30 fold using an Amicon ultrafiltration assembly with a YM-30 membrane.

Checkerboard analysis was performed to assess the random (chemokinetic) versus the directed

(chemotactic) nature of the motility response to ATX. Chambers were assembled with different concentrations of ATX above and below the filter, using ATX purified through the weak anion exchange fractionation step. Squares below the diagonal reflect response to a positive gradient, squares above reflect response to a negative gradient, and squares on the diagonal reflect random motility in the absence of a gradient. ATX stimulates both chemotactic and chemokinetic responses (Figure 10), with chemotactic responses as high as fifteen-fold above background and chemokinesis as high as eight-fold above background.

Amino acid analysis after complete acid hydrolysis was used to quantitate purified protein. This hydrolysis was carried out on protein excised from a polyacrylamide gel and presumed to be pure. The analysis indicated that 2.7 nmol of protein was present after fractionation on the molecular sieve. After fractionation by strong anion exchange chromatography, approximately 300 pmol remained. The results of the analysis are shown in Table 3.

TABLE 3. AMINO ACID COMPOSITION OF AUTOTAXIN
(CYS and TRP were not determined in this analysis)

Amino Acid	Residues/100
ASX	12.5
THR	6.0
SER	5.7
GLX	9.4
PRO	7.4
GLY	7.0
ALA	3.9
VAL	6.7
MET	1.2
ILE	4.3
LEU	9.0
TYR	5.2
PHE	5.2
HIS	3.8
LYS	7.4
ARG	5.4

EXAMPLE 3

ATX Degradation and Determination of Amino Acid Sequence

Attempts to obtain N-terminal sequence information from purified ATX repeatedly proved futile. The purified protein was therefore, sequentially digested and the resulting peptides fractionated by reverse phase chromatography. The results are shown in Figure 11. Multiple sharp peaks including clusters at both the hydrophilic and hydrophobic ends of the gradient are seen.

Several of these peptide peaks were chosen randomly for Edman degradation and N-terminal amino acid sequence analysis. Seven of the eight peaks (shown in Figure 11) chosen gave clear single sequence information as seen in Table 4. Using material from a separate digestion and purification, the remaining four sequences were also obtained.

Separate sense and antisense oligonucleotide probes were synthesized according to the fragment sequences of Table 4 by methods known to one skilled in the art. Representative probes are shown in Table 5.

TABLE 4. Peptide sequences for Autotaxin.

PEPTIDE NO.	AMINO ACID SEQUENCE	SEQ ID: NO:	NAME
1.	WHVA	SEQ ID NO:1	ATX 18
2.	PLDVYK	SEQ ID NO:2	ATX 19
3.	YPAFK	SEQ ID NO:3	ATX 20
4.	QAEVS	SEQ ID NO:4	ATX 24
5.	PEEVTRPNYL	SEQ ID NO:5	ATX 29
6.	YDVPWNETI	SEQ ID NO:6	ATX 47
7.	VPPFENIELY	SEQ ID NO:7	ATX 48
8.	GGQPLWITATK	SEQ ID NO:8	ATX 100

9.	VNSMQTVFVGY-GPTFK	SEQ ID NO:9	ATX 101
10.	DIEHLTSLDFFR	SEQ ID NO:10	ATX 102
11.	TEFLSNYLTNVDD-ITLVPETLGR	SEQ ID NO:11	ATX 103
12.	QYLHQYGSS	SEQ ID NO:26	ATX 37
13.	VLNYF	SEQ ID NO:27	ATX 39
14.	YLNAT	SEQ ID NO:28	ATX 40
15.	HLLYGRPAVLY	SEQ ID NO:29	ATX 41
16.	SYPEILTPADN	SEQ ID NO:30	ATX 44
17.	XYGFLFPPYLSSSP	SEQ ID NO:31	ATX 53
18.	TFPNLYTFATGLY	SEQ ID NO:32	ATX 59
19.	VNVISGPIFDYDYDGLH DTEDK	SEQ ID NO:33	ATX 104

Peptide numbers 1-7 refer to peaks numbered in Figure 11. Peptide numbers 12-18 refer to peptides purified from the preparation which yielded peptide numbers 1-7. Peptides 8-11 and 19, are from a separate purification, not shown in Figure 11.

X refers to potentially glycosylated residues.

TABLE 5.

Oligonucleotides synthesized from peptide sequences of autotaxin (ATX). The number of the oligonucleotide corresponds to the ATX peptide number as per Table 4. The final letter suffix distinguishes whether the oligonucleotide is a sense (S) or antisense (A) sequence.

Oligo	Sequence	SEQ ID NO:
A-18A	GTT-GGC-AGC-NAC-RTG-CCA	SEQ ID NO:12
A-18S	TGG-CAY-GTN-GCT-GCC-AAC	SEQ ID NO:13
A-20A	CTT-GAA-GGC-AGG-GTA	SEQ ID NO:14
A-20S	TAY-CCT-GCN-TTY-AAG	SEQ ID NO:15
A-29A	GGT-NAC-YTC-YTC-AGG	SEQ ID NO:16

A-29S	CCT-GAR-GAR-GTN-ACC	SEQ ID NO:17
A-47A	NGT-NGC-RTC-RAA-TGG-CAC-RTC	SEQ ID NO:18
A-47S	GAY-GTG-CCA-TTY-GAY-GCN-ACN	SEQ ID NO:19
A-48A	GTT-DAT-RTT-STC-RAA-TGG-GGG	SEQ ID NO:20
A-48S	CCC-CCA-TTT-GAG-AAC-ATC-AAC	SEQ ID NO:21
A-100A	CTT-NGT-NGC-NGT-DAT-CCA-NAR-GGG-YTG-GCC-GCC	SEQ ID NO:22
A-100S	GGC-GGC-CAR-CCC-YTN-TGG-ATH-ACN-GCN-ACN-AAG	SEQ ID NO:23
A-101A	CTT-RAA-GGT-GGG-GCC-RTA-GCC-CAC-RAA-GAC-TGT-YTG-CAT	SEQ ID NO:24
A-101S	ATG-CAR-ACA-GTC-TTY-GTG-GGC-TAY-GGC-CCC-ACC-TTY-AAR	SEQ ID NO:25

EXAMPLE 4

Antipeptide Antibodies

Rabbits were injected with ATX-101 (SEQ ID NO:10) which had been cross-linked to bovine serum albumin. Antisera from these rabbits was subjected to salt precipitation followed by purification using affinity chromatography with Affi-Gel 10 beads covalently linked to the peptide, ATX-101 (SEQ ID NO:10). This affinity purified antibody reacted with the partially purified protein on immunoblots. This same antibody has been used to perform immunohistochemical stains on human tissue.

EXAMPLE 5

Enzymatic Deglycosylation of ATX

Purified ATX that was to be treated with peptide N-glycosidase F (PNGase F) was first dialyzed into 0.2 M sodium phosphate, 10% (v/v) ethylene glycol pH 7.0, using Centricon-30 ultrafiltration tubes. Varying concentrations of PNGase F were added to the ATX and incubated 16-18 hr. at 37°C. Complete digestion appeared

to occur at concentrations of enzyme above 30 mU/ml (where 1 U converts 1mmol of substrate/min). For comparison, the experiments were repeated in the presence of 0.1 M β-mercaptoethanol or 0.1% (w/v) SDS plus 0.5% (v.v) Nonidet-P40. ATX that was to be treated with neuraminidase or O-glycosidase was dialyzed into 20 mM sodium phosphate, 0.1 M calcium acetate, and 10% (v/v) ethylene glycol (pH 7.2). Neuraminidase was added to a final concentration of 2 U/ml. For treatment with neuraminidase alone, this mixture was incubated 16-18 hr at 37°C. Since O-glycosidase requires the removal of terminal sialic acid residues for efficient deglycosylation, ATX was pre-incubated with neuraminidase for 30-125 mU/ml and incubated 16-18 hr. at 37°C. The treated ATX was then dialyzed into 50 mM Tris with 20% ethylene glycol for storage at 5%C.

Treatment of ATX with N-glycosylation altering agents

A2058 cells were split into four 150 cm^2 flasks and incubated until just subconfluent in DMEM supplemented with 10% fetal calf serum. The medium was then replaced with fresh 10% FCS/DMEM to which had been added DPBS for control, 1mM dMAN, 1 mM NMdNM, or 10 mM (1.7 mg/ml) Swn. Concentrations of these pharmacological agents were similar to those previously described as inhibiting N-glycan processing enzymes in melanoma cells (Seftor, et al. 1991; Dennis, et al. 1990) as well as carcinoma cells (Ogier, et al. 1990). On the next day, each flask was washed twice with Dulbecco's phosphate buffered saline with calcium ("DPBS") then 20 ml of Dulbecco's minimum essential medium ("DMEM") supplemented with 0.01% (w/v) bovine serum albumin ("BSA") was added. The same concentration of each agent was added to the appropriate equilibrated flask and incubated for ~ 24 hr, after which the medium from each treatment group was collected, concentrated, washed into DPBS and stored at 5°C.

Cells from each flask were trypsinized and

counted. There was no loss of viability or reduced cell number in any of the treatment groups compared to control cells.

Effect of PNGase F on ATX

ATX binds to concanavalin A ("Con A") agarose beads and is eluted with buffer containing 0.5 M methyl a-D-mannopyranoside, indicating that ATX is likely to contain mannose residues. Such mannose sugar residues are most characteristic of N-linked oligosaccharides. In order to verify that ATX contained asparagine-linked oligosaccharides, we treated it with the endoglycosidase, PNGase F, which cleaves high mannose, hybrid, and complex N-linked oligosaccharides at the asparagine residue.

Partially purified ATX was treated with 60 mU/ml of enzyme under a variety of increasingly denaturing conditions and then separated by polyacrylamide gel electrophoresis (Figure 16). Lane 1 shows untreated material; the 125 kDA band (arrow) is autotaxin. When this material is treated overnight with PNGase F under very mild conditions, the size of the 125 kDa band decreases to ~100- 105 kDa. Addition of 0.1 M b-mercaptoethanol (Lane 2) or 0.5% Nonidet-P40 (lane 3) to the ATX sample has no effect on the size of the resultant protein band. Even complete denaturation of ATX of boiling the sample for 3 min in 0.1% SDS with (lane 5) or without (lane 4) β-mercaptoethanol, followed by addition of 0.5% Nonidet-P40 to maintain enzymatic activity, has no effect on the final size of deglycosylated protein, indicating that the deglycosidation reaction was complete even under mild conditions.

Because these results showed that ATX contained N-linked oligosaccharide groups, it became important to see if these sugar moieties were necessary for stimulation of motility. The partially purified ATX sample was treated with varying concentrations of PNGase F (0.1 to 60 mU/ml) under mild, non-denaturing conditions. Analysis of

the resulting digest by polyacrylamide gel electrophoresis is shown in Figure 17A. As this figure shows, the digestion was incomplete using from 0.1 to 10 mU/ml of enzyme and resulted in a smear of protein between 100-125 kDa. However, at higher concentrations of enzyme, cleavage of N-linked oligosaccharides from ATX appears to be complete. When these different digestion products were compared for their capacity to stimulate motility (Figure 17B), there was no significant difference between groups.

EXAMPLE 6

Cloning the 3' end of Autotaxin (4C11)

ATX is active in picomolar to nanomolar concentrations and is synthesized in very small concentrations by A2058 cells. As might be expected, the cDNA clone was relatively rare, requiring various strategies and multiple library screenings in order to identify it (Figure 12). Attempts to utilize degenerate oligonucleotides deduced from known peptide sequences were unsuccessful--whether we used the oligo nucleotides for screening cDNA libraries or for reverse transcription of mRNA followed by amplification with the polymerase chain reaction (RT/PCR). We then utilized an affinity-purified anti-peptide ATX-102 antibodies to screen an A2058 expression library.

These anti-peptide antibodies were generated by methods well established in the art and described previously with slight modification (Wacher, et al., 1990). In brief, the previously identified peptide, ATX-102 (Stracke, et al., 1992), was synthesized on a Biosearch 9600 peptide synthesizer. It was then solubilized in 1X PBS containing 20% (v/v) DMSO and conjugated to the protein carrier, bovine serum albumin (BSA), with glutaraldehyde. For the first injection into New Zealand white rabbits, the BSA-peptide conjugate was emulsified with complete Freund's adjuvant and injected subcutaneously. For subsequent injections, the BSA-

peptide conjugate was emulsified with incomplete Freund's adjuvant. The resultant antiserum was heat-inactivated at 56°C for 30 min. Immunoglobulins were precipitated out in 47% saturated ammonium sulfate, then redissolved and dialyzed into PBS. Antibodies were adsorbed onto peptide-conjugated Affi-Gel 10 resin (made using the BioRad protocol), eluted with 0.1 N acetic acid, and neutralized with 2 M Tris-HCl, pH 8. The resulting affinity-purified antibodies were dialyzed into DPBS, concentrated, aliquotted, and stored at -20°C. The antibodies were found to recognize a 125 kDa protein on immunoblots of partially purified A2058 conditioned medium and to preferentially stain some breast carcinoma cells compared to normal breast using immunohistochemical techniques.

An A2058 cDNA library was prepared by purifying poly-A purified mRNA from the cells then size-selecting mRNA > 1000 bp for the preparation of cDNA. The cDNA inserts were placed into λgt11 directionally, using the ProMega cDNA kit using standard methods well-established in the field. LE 392 cells were infected with the λgt11 and plaques were transferred onto nitrocellulose membranes by overnight incubation at 37°C. The antibody was incubated with the membranes in blocking buffer for 2 hr at room temperature, using approximately twice the concentration of antibody which gave a strong response on Western blot analysis. Secondary antibody was goat anti-rabbit immunoglobulin, and the blot was developed colorimetrically with 4-chloro-1-naphthol.

Positive clones were confirmed by antibody competition with specific peptides but not unrelated peptides. Using this technique and multiple subclonings, we obtained a partial cDNA clone of the autotaxin gene, which we called 4C11. The 4C11 insert was removed from λgt11 by restriction enzyme digests and subcloned into pBluescript for sequencing by standard Sanger techniques (Sanger, et al., 1977). The 4C11 clone contained bases,

including the poly-adenylated tail and the AATAAA adenylation signal locus, i.e., it contained the 3' terminus of the gene. It also included a 627 base open reading frame. Database analysis of this nucleotide sequence revealed that it is unique. The predicated amino acid sequence for 4C11 is 209 amino acids long with exact matches for 7 previously identified ATX peptides: (ATX-20, ATX-34, ATX-102, ATX-104, ATX-204, ATX-215, and ATX-244).

EXAMPLE 7

Cloning the 5' terminus of ATX

Database analysis of the 3' terminus of the ATX gene demonstrated a novel protein. However, we have found an interesting homology that has helped to guide us in exploring its function. ATX had a 45% amino acid identity and a 57% nucleotide identity with PC-1, a marker of B cell activation found on the surface of plasma cells. Using the PC-1 protein sequence as a guide, we found that ATX peptide homologies were scattered throughout the length of the protein. The only exception was the far amino terminus of PC-1, which includes the transmembrane and intracellular domains, and which had no homologies. Knowing approximate localization of the ATX peptides along the length of ATX, we then amplified different segments of ATX by the PCR (Figure 13). These amplified segments of DNA were then subcloned into plasmids utilizing the TA Cloning kit of ProMega. The PCR amplified DNA could then be sequenced using standard Sanger sequencing techniques (Sanger, et al., 1977).

Cloning of full length ATX gene

A reverse transcriptase reaction was performed using total or oligo-(dT) purified RNA from A2058 or N-tera 2D1 cells as template and an anti-sense primer from the 5' end of 4C11 (GCTCAGATAAGGAGGAAAGAG). This was followed by one or two PCR amplification of the resultant cDNA using the commercially available kit from Perkin-Elmer and following manufacturer's directions.

These PCR reactions utilized nested antisense primers from 4C11 (GAATCCGTAGGACATCTGCTT and TGTAGGCCAAACAGTTCTGAC) as well as degenerate, nested sense primers deduced from ATX peptides: ATX-101 (AAYTCIATGCARACIGTITTYGTIG and TTYGTIGGITAYGGICCIACITTYAA), ATX-103 (AAYTAYCTIACIAAYGTIGAYGAYAT and GAYGAYATIACICTIGTICCIGGIAC), or ATX-224 (TGYTTYGARYTICARGARGCIGGICCICC). The amplified DNA was then purified from a polyacrylamide gel using standard procedures and ligated into the pCR™ plasmid using the TA cloning kit (Invitrogen Corporation) according to manufacturer's directions.

The 5′ RACE kit was utilized to extend the 5′ end of ATX cDNA using total RNA from N-tera 2D1 as template and previously obtained sequence as primer (GCTGTCTTCAAACACAGC). The 5′ end of the A2058 synthesized protein was obtained by using previously obtained sequence as primer (CTGGTGGCTGTAATCCATAGC) in a reverse transcriptase reaction with total A2058 RNA as template, followed by PCR amplification utilizing the 5′ end of N-tera 2D1 sequence as sense primer (CGTGAAGGCAAAGAGAACACG) and a nested antisense primer (GCTGTCTTCAAACACAGC). A2058 DNA encoding ATX is set forth in a SEQ ID NO:68 and the amino acid sequence is provided in SEQ ID NO:69.

DNA sequencing: DNA sequencing was performed using dideoxy methodology (Sanger, et al. 1977) and (^{35}S)dATP (Du Pont, New England Nuclear).

We have found one region between the 5′ end of the 4C11 and the ATX peptide designated ATX-101, also referred to as the "hot spot". This region has been sequenced five times with different sequences found each time. The hot spot appears to be located within the region from approximately nucleotide 1670 to 1815. The consensus sequence is represented by amino acids position 559 through 604. Variations found include DNA sequence

that results in single and multiple amino acid insertions. One sequence had a stop codon in this region and may have represented an intron. This region has been found to be variable in forms of ATX.

EXAMPLE 8

Cloning ATX in a human teratocarcinoma cell line

The fact that ATX is present in other cancer cells was confirmed by sequence information from N-tera 2D1, a human teratocarcinoma cell line. For these cells, a prepared cDNA library in λgt10 was amplified and the cDNA inserts were extracted. Using oligonucleotide primers based on known A2058 ATX sequence, DNA segments were amplified by PCR. The DNA segments were then subcloned into plasmids and sequenced as for A2058. We have 3104 bp DNA sequence for N-tera ATX (SEQ ID NO:66) and smaller portions thereof. This includes an open reading frame that codes for a putative protein containing 861 amino acids (SEQ ID NO:67) and smaller portions thereof. Like the A2058 ATX, the N-tera 2D1 sequence has homologies for multiple ATX peptides (Figure 15). Sequence homology between the A2058 and N-tera 2D1 cells is approximately 99%.

EXAMPLE 9

Cloning 5' end of ATX in human normal liver

The 5' end of ATX has proven difficult to obtain from either tumor cell line to date. Normal human liver mRNA was therefore amplified using the 5' RACE kit (Clontech) with known sequence from A2058 ATX as antisense primer. A DNA segment was obtained and has been sequenced. This segment codes for 979 amino acids, including an initiating methionine (SEQ ID NO:38). The putative protein sequence also includes a 20 amino acid transmembrane domain which is different from the tumor ATX's (SEQ ID NO:54), as shown in Table 7. Both tumorous forms of ATX apparently lack a transmembrane region and are instead secreted proteins.

Table 7

Nucleotide and Amino Acid Sequences Encoding Liver ATX Amino Terminus containing the Transmembrane region

Protein Sequence (SEQ ID NO: 54)

Met Ala Arg Arg Ser Ser Phe Gln Ser Cys Gln Asp Ile Ser Leu Phe Thr Phe Ala Val Gly Val Asn Ile Cys Leu Gly Phe Thr Ala His Arg Ile Lys Arg Ala Glu Gly Trp

DNA Sequence (SEQ ID NO: 53)

ATGGCAAGGA GGAGCTCGTT CCAGTCGTGT CAAGATATAT CCCTGTTCAC
TTTTGCCGTT GGAGTCAATA TCTGCTTAGG ATTCACTGCA CATCGAATTA
AGAGAGCAGA AGGATGG

EXAMPLE 10

Domains of ATX

Searches of protein databases (Pearson, et. al. 1988) confirmed that the homology between ATX and PC-1 was present throughout the length of the extracellular portion of the molecules (Buckley, et. al., 1990; Funakoshi, et. al. 1992). There is a 45% amino acid identity and a 64% similarity between the 2 protein sequences (Fig. 18). For the cDNA sequence, the identity is ~57%.

These proteins share several interesting properties and domains (Fig. 19). Both have a number of potential N-linked glycosylation sites: four for ATX (Asn54, Asn463, Asn577, Asn859) and nine for PC-1. Both have adjacent somatomedin B domains near the amino end of the extracellular domain. This somatomedin B domain is a cysteine-rich region containing 3 presumed cystine cross-

linkages. ATX has 33 Cys residues and PC-1 has 37; 30 of these Cys residues are identical in placement. Both proteins also contain the loop region of an EF hand (Buckley, et. al. 1990; Kretsinger, 1987). In addition, both proteins have a transmembrane/signal peptide region with a short intracellular peptide, common in ectoenzymes (Maroux, 1987). However, the amino acid identity between ATX and PC-1 in the intracellular and transmembrane regions is only 11%.

Finally, both proteins have a region homologous to the bovine intestinal phosphodiesterase enzymatic domain with conversation of the threonine that is thought to act as the intermediate phosphate binding site (Culp, et al. 1985). PC-1 has been demonstrated to have phosphodiesterase type I, nucleotide pyrophosphatase, and threonine-specific kinase enzymatic activities (Rebbe, et al. 1991; Oda, et al. 1991). In order to test whether purified ATX had type I phosphodiesterase activity, samples were incubated with p-nitrophenyl thymidine-5'-monophosphate at pH 8.9 for 30 min. Samples were assayed in a 100 μl volume containing 50 mM Tris-HCl, pH 8.9 and 5 mM p-nitrophenyl thymidine-5'-monophosphate. After a 30 minute incubation at 37 °C the reactions were terminated by addition of 900 ml 0.1 N NaOH and the amount of product formed was determined by reading the absorbance at 410 nm. ATX was found to hydrolyze the p-nitrophenyl thymidine-5'-monophosphate (Razzell, 1963) at a rate of 10 pmol/ng/min, a reaction rate similar to that reported for PC-1 (Oda, et al. 1993).

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All publications mentioned hereinabove are hereby incorporated in their entirety by reference.

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be appreciated by one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the present invention and appended claims.

CLAIMS:

1. A DNA segment coding for a polypeptide comprising an amino acid sequence corresponding to autotaxin, or a fragment thereof having at least 5 amino acids.

2. The DNA segment according to claim 1, wherein said DNA segment encodes the amino acid sequence selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:11, SEQ ID NO:26 through SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38 through SEQ ID NO:52, SEQ ID NO:66 and SEQ ID NO:69.

3. An isolated polypeptide comprising an amino acid sequence corresponding to autotaxin, or a fragment thereof having at least 5 amino acids.

4. The polypeptide according to claim 3, wherein said amino acid sequence comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:11, SEQ ID NO:26 through SEQ ID NO:34, SEQ ID NO:36, and SEQ ID NO:38 through SEQ ID NO:52, SEQ ID NO:67 and SEQ ID NO:69.

5. An isolated polypeptide bound to a solid support, comprising an amino acid sequence corresponding to autotaxin, or a fragment thereof having at least 5 amino acids.

6. The polypeptide according to claim 5, wherein said polypeptide comprises the amino acid sequence selected from the group consisting of the SEQ ID NO:1 through SEQ ID NO:11, SEQ ID NO:26 through SEQ ID NO:34, SEQ ID NO: 36, SEQ ID NO:38 through SEQ ID NO:52., SEQ ID NO:67 and SEQ ID NO:69.

7. A recombinant DNA molecule comprising a vector and the DNA segment according to claim 1.

8. A cell that contains the recombinant DNA molecule according to claim 7.

9. An antibody having binding affinity for autotaxin, or binding fragment thereof.

10. A method of producing a recombinant autotaxin polypeptide said method comprising:

culturing a cell containing the recombinant DNA molecule of claim 7 under conditions such that the DNA segment is expressed, producing said polypeptide; and

isolating said polypeptide.

11. A method of purifying the autotaxin peptide of claim 3, comprising the steps of:

i) collecting and concentrating supernatant from cultured A2058 human melanoma cells whereby a first preparation of said peptide is produced;

ii) salt fractionating said first preparation to produce a second peptide preparation;

iii) isolating said peptide from said second preparation so that said peptide is obtained in substantially pure form.

12. The method of claim 11, wherein said isolating step is effected by column chromatography.

13. An isolated DNA encoding an autotaxin protein or fragment thereof wherein said DNA includes a nucleic acid sequence selected from the group consisting of SEQ ID NO:35, SEQ ID NO:37 and SEQ ID NO:38.

14. The DNA segment according to claim 1, wherein said DNA fragment comprises any one of the SEQ ID NO:12 through SEQ ID NO:25, or SEQ ID NO:39 through SEQ ID NO:52.

15. The DNA segment according to claim 13 wherein said DNA segment comprises any one of the SEQ ID NO:12 through SEQ ID NO:25.

16. An isolated polypeptide comprising an amino acid sequence corresponding to autotaxin.

17. A polypeptide bound to a solid support and comprising an amino acid sequence corresponding to autotaxin.

18. A recombinant autotaxin polypeptide

according to claim 3.

19. An isolated polypeptide according to claim 3 having cell motility activity.

ABSTRACT OF THE DISCLOSURE

The present invention relates, in general, to autotaxin. In particular, the present invention relates to a DNA segment encoding autotaxin; recombinant DNA molecules containing the DNA segment; cells containing the recombinant DNA molecule; a method of producing autotaxin; antibodies to autotaxin; and identification of functional domains in autotaxin.

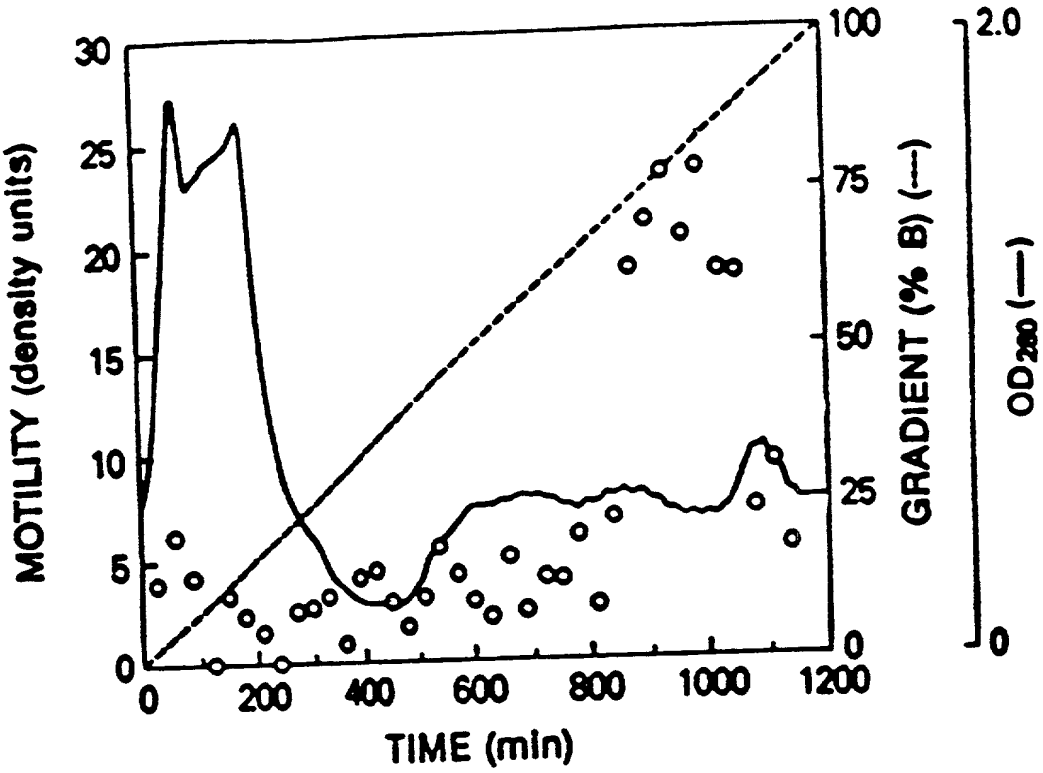
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FIGURE 1

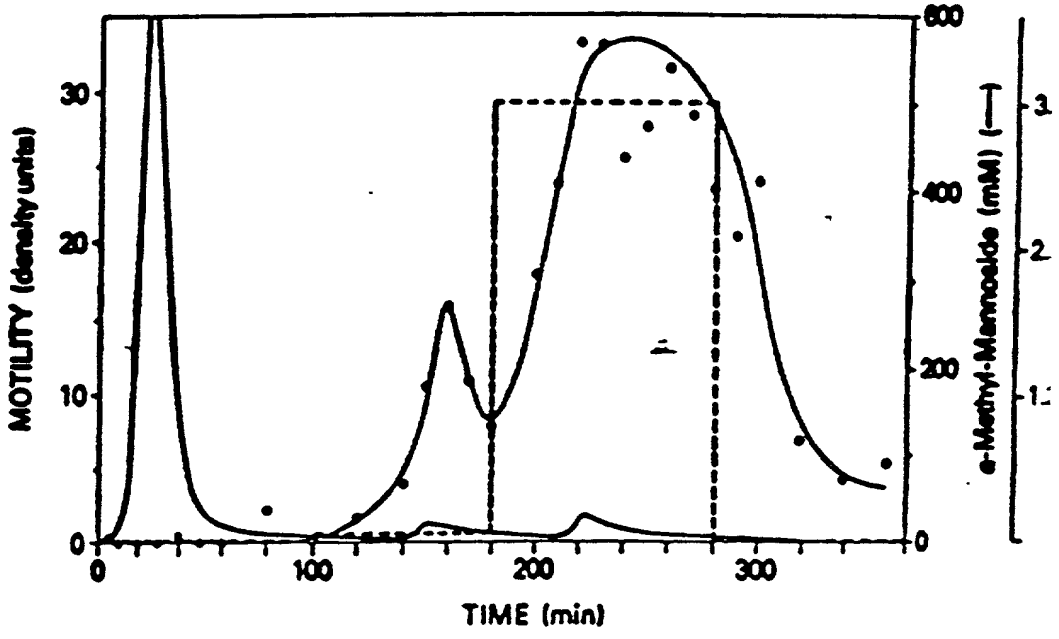
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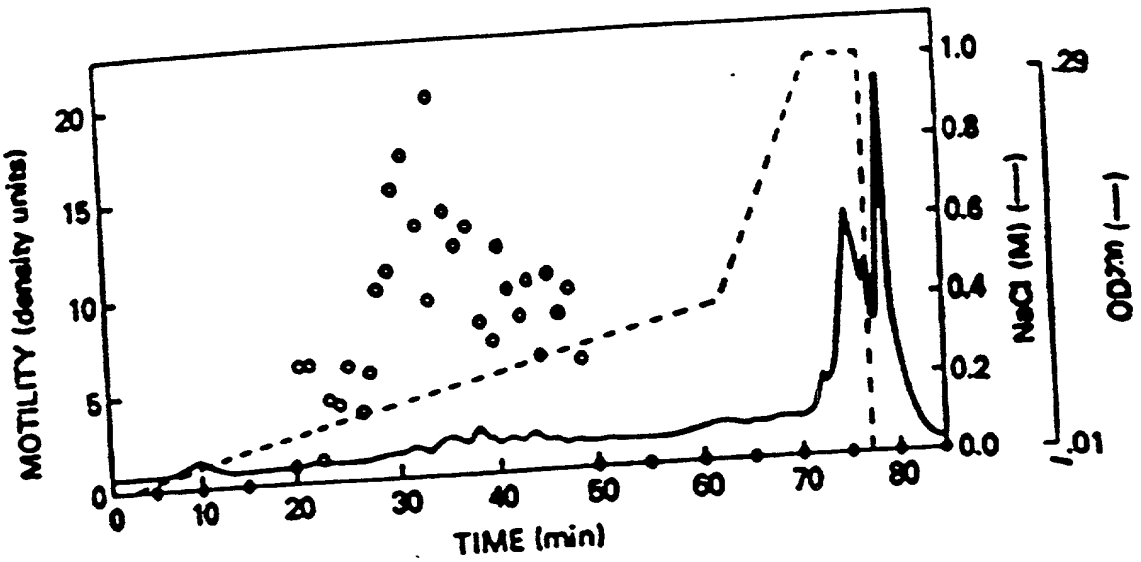
FIGURE 2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40
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60
70
80
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0.2
0.4
0.6
0.8
1.0
.01
.29

FIGURE 3

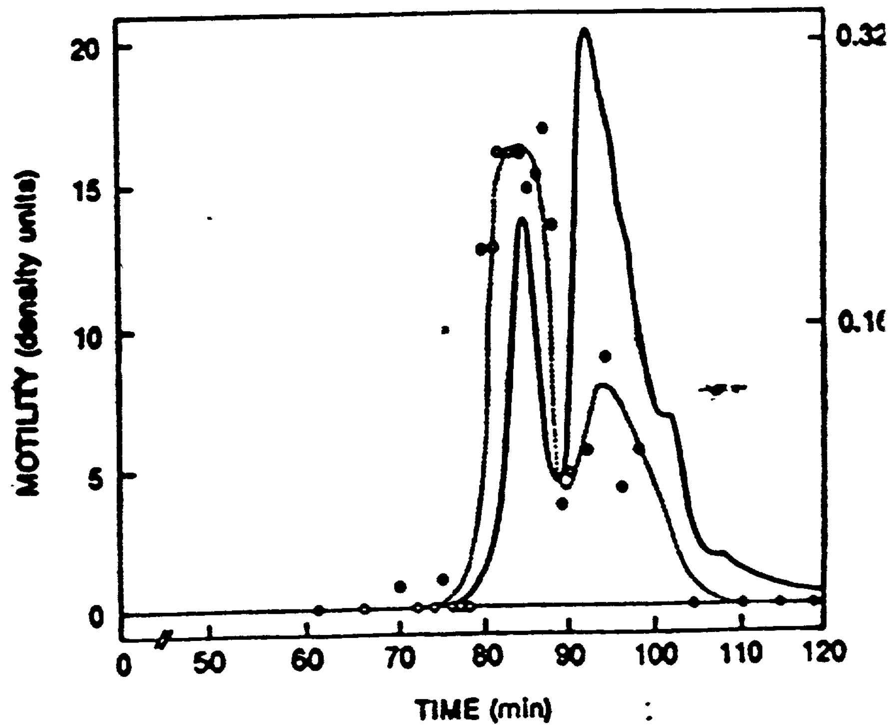


FIGURE 4

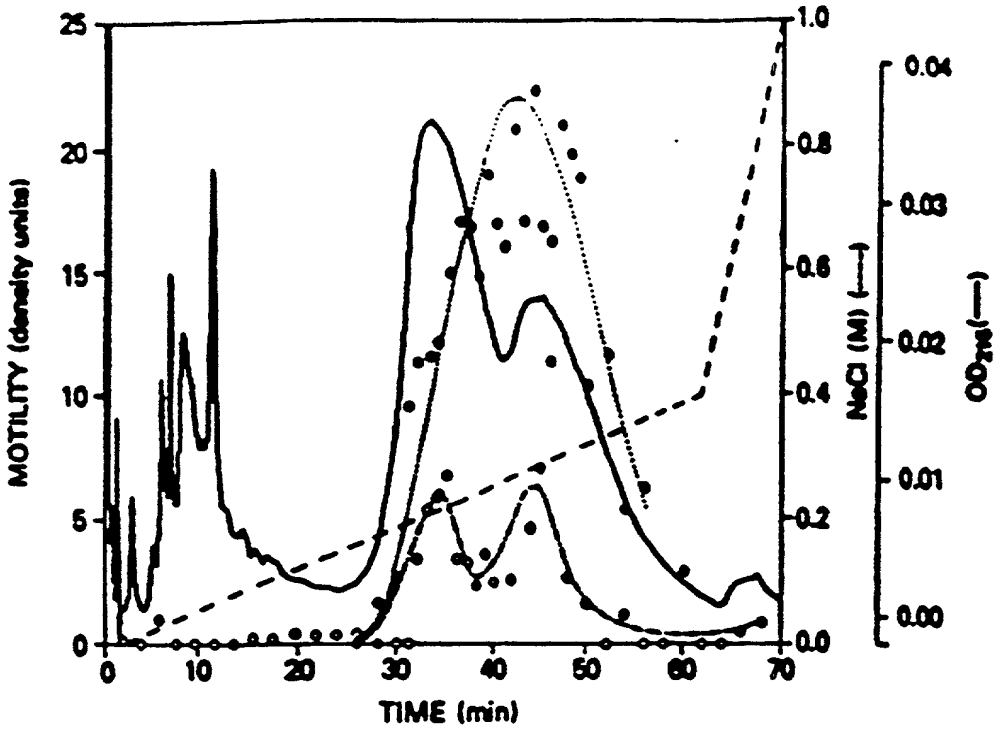

MOTILITY (density units)
TIME (min)
NaCl (M) (——)
OD546 (——)
0
5
10
15
20
25
0
10
20
30
40
50
60
70
0.0
0.2
0.4
0.6
0.8
1.0
0.00
0.01
0.02
0.03
0.04

FIGURE 5

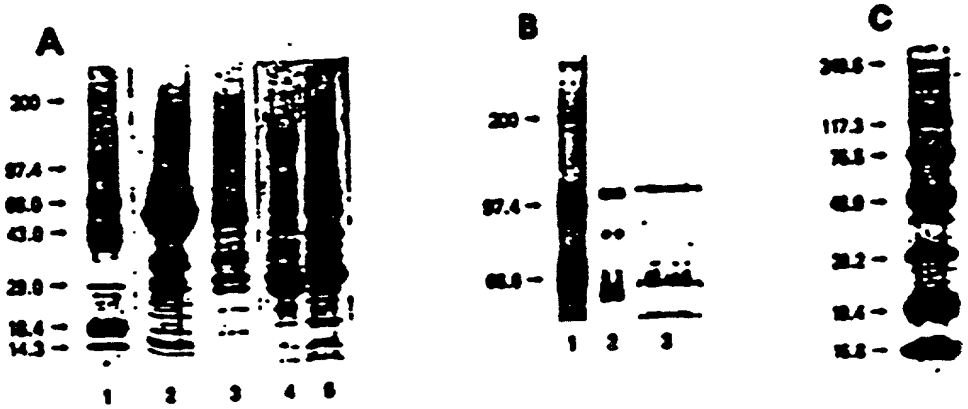


FIGURE 6

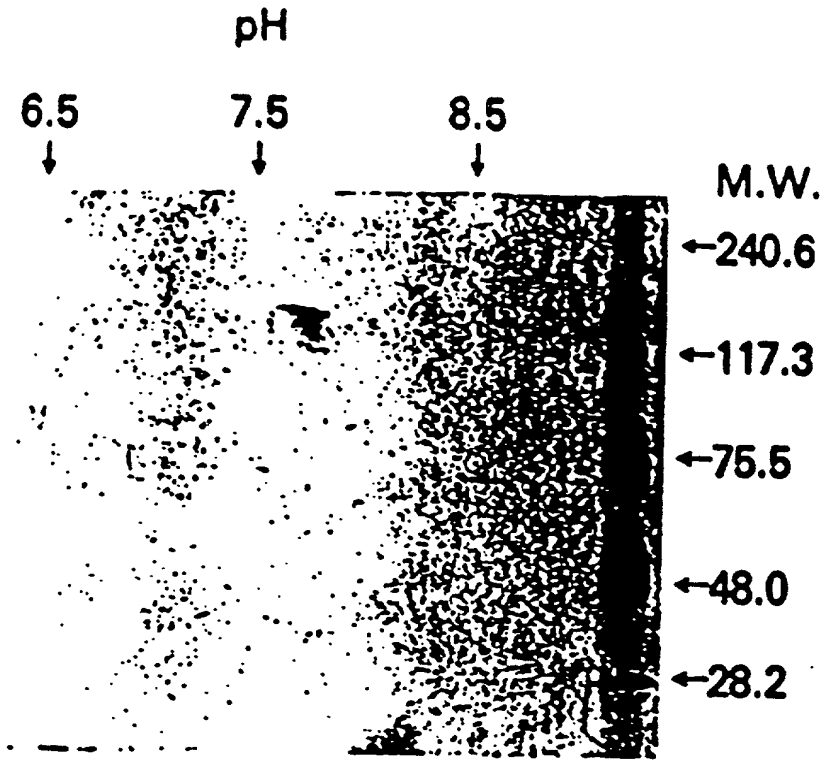


FIGURE 7

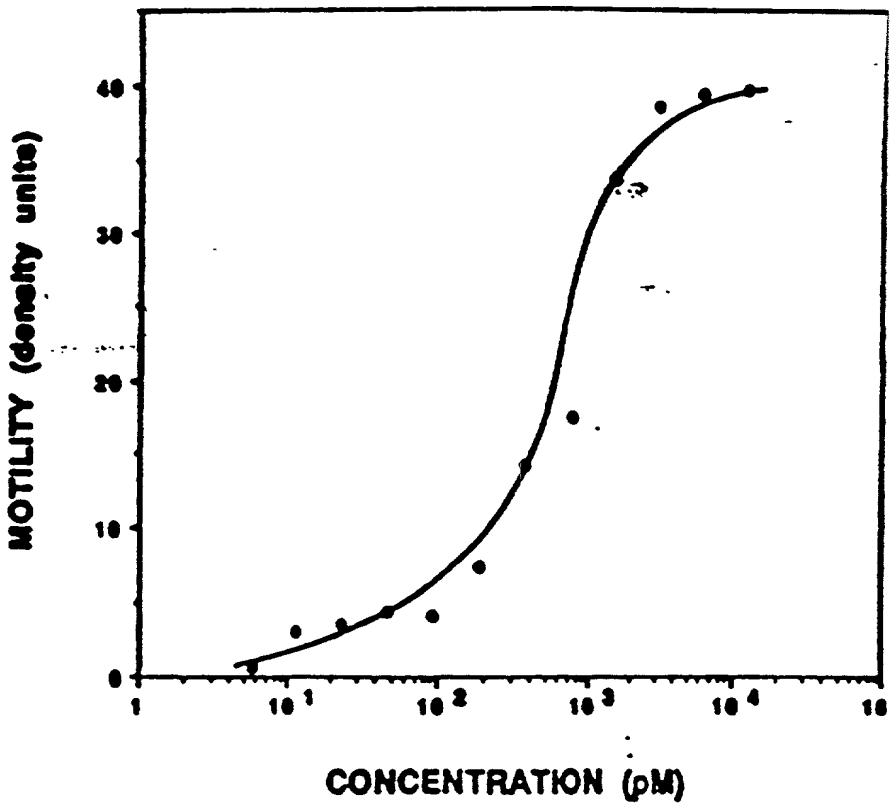


FIGURE 8

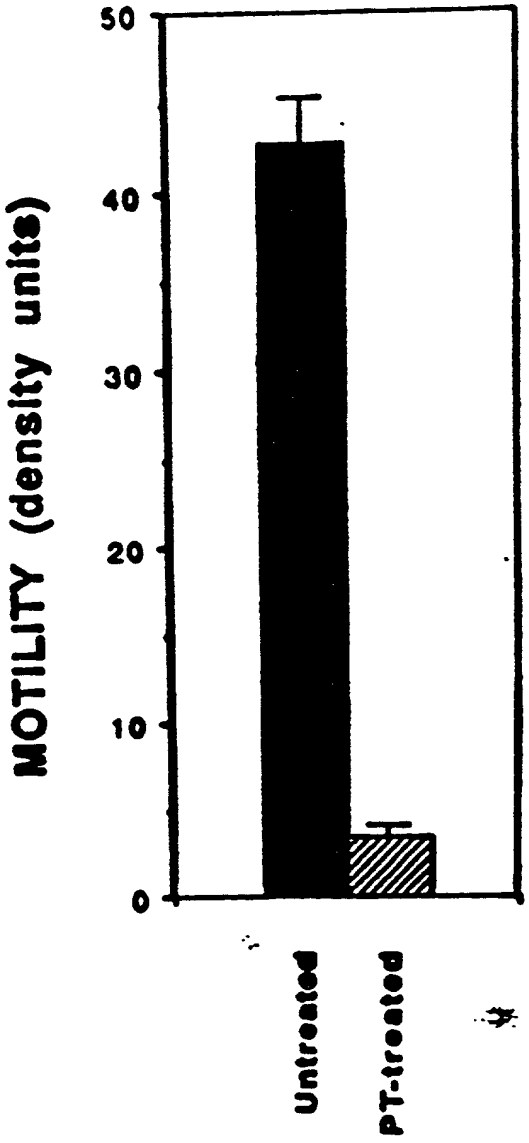


FIGURE 9

FIG. 10

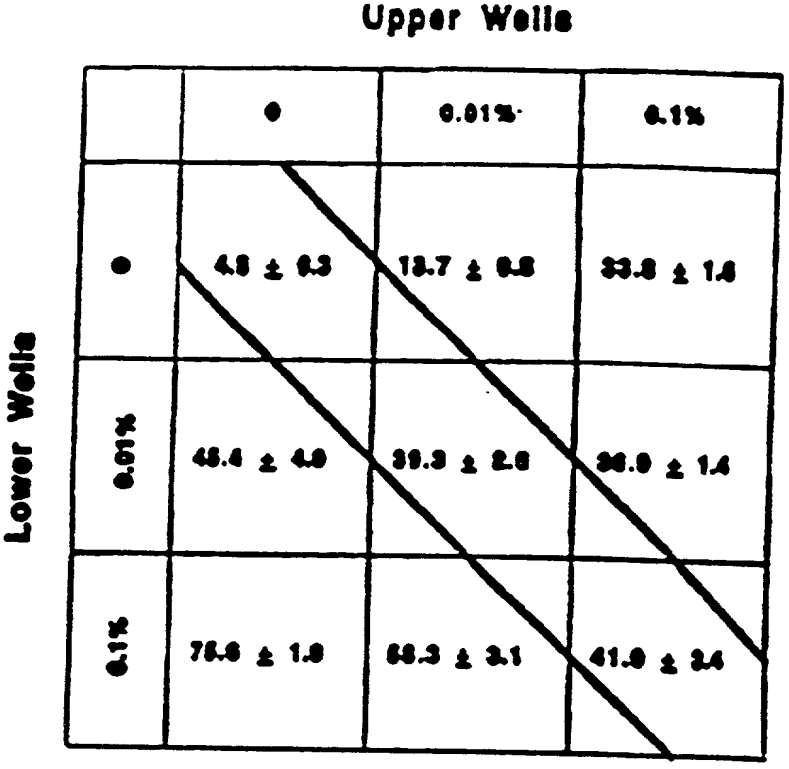
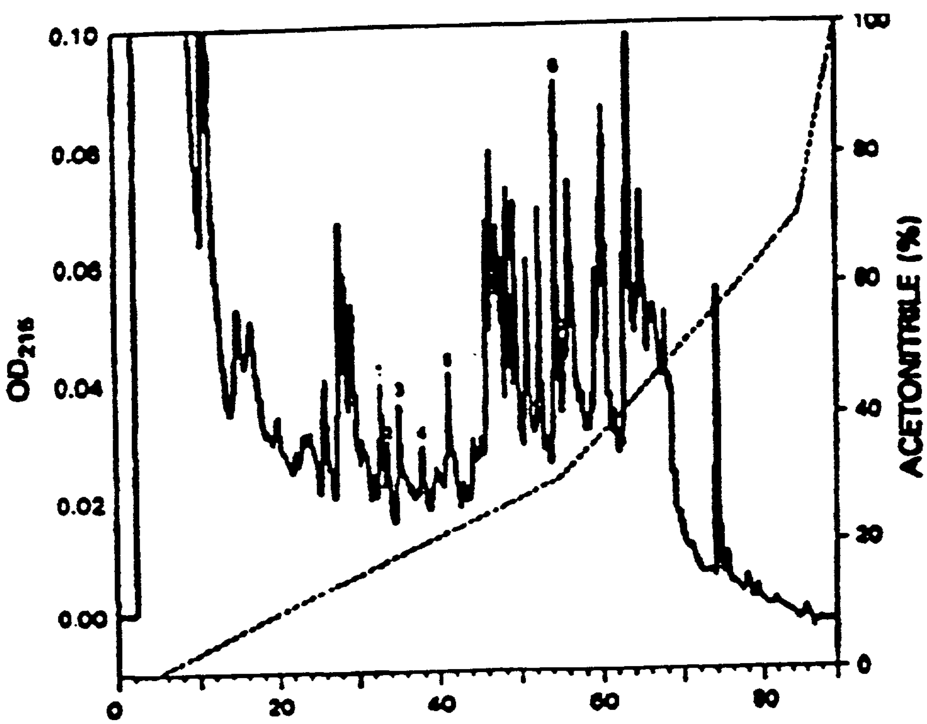


FIG. 11



TIME (min)

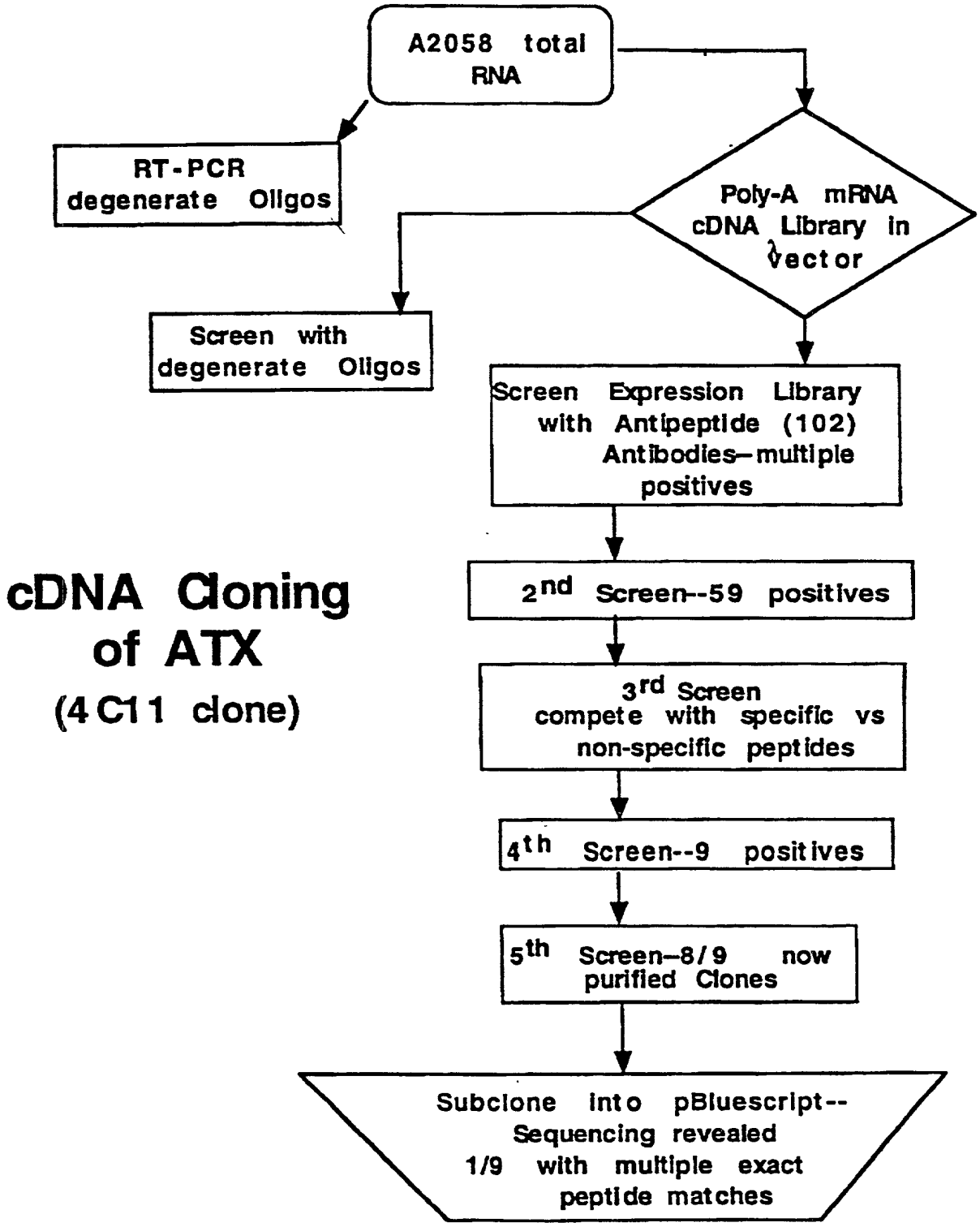

A2058 total RNA
RT-PCR degenerate Oligos
Poly-A mRNA cDNA Library in Vector
Screen with degenerate Oligos
Screen Expression Library with Antipeptide (102) Antibodies–multiple positives
2nd Screen--59 positives
3rd Screen compete with specific vs non-specific peptides
4th Screen--9 positives
5th Screen–8/9 now purified Clones
Subclone into pBluescript--Sequencing revealed 1/9 with multiple exact peptide matches
cDNA Cloning of ATX
(4C11 clone)

FIGURE 12

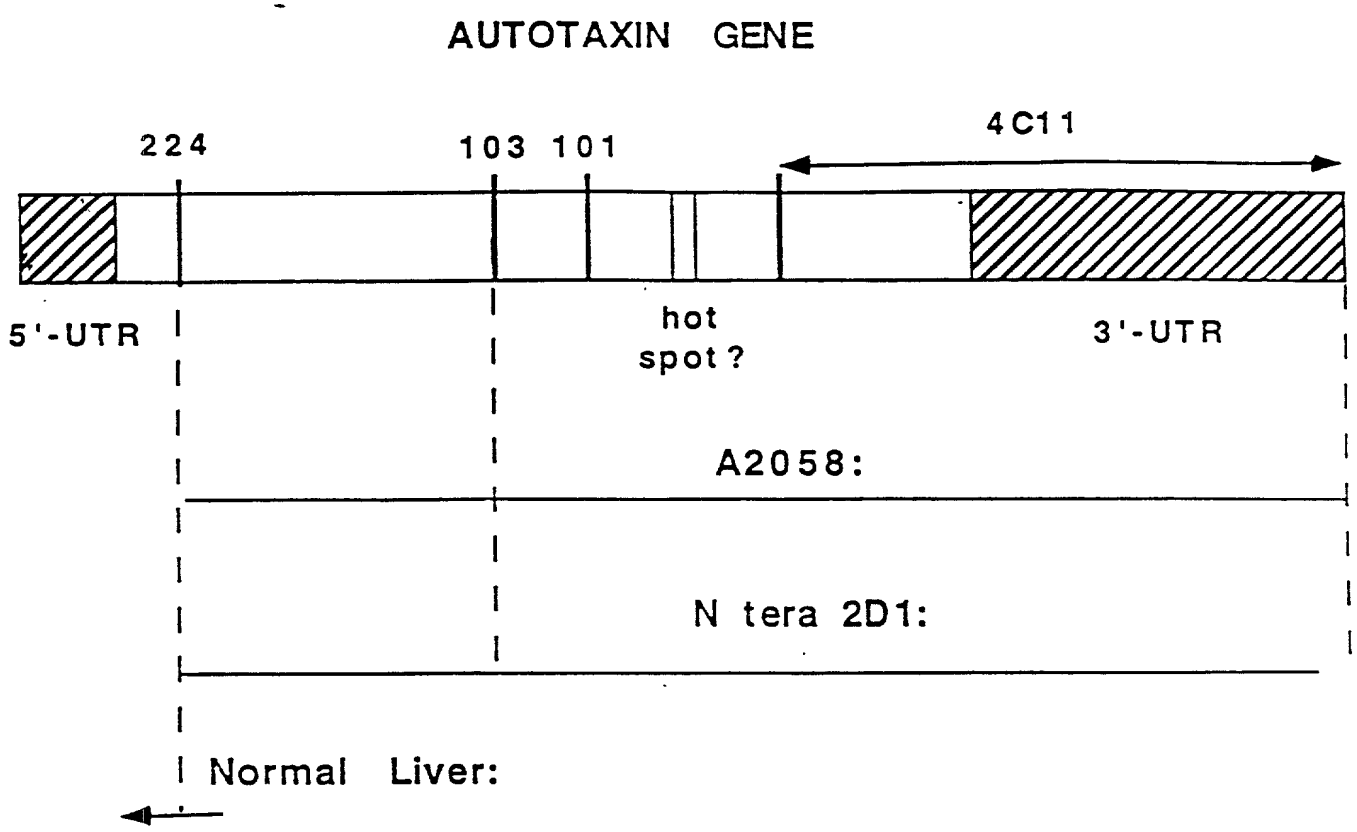

AUTOTAXIN GENE
224
103 101
4C11
5'-UTR
hot
spot?
3'-UTR
A2058:
N tera 2D1:
Normal Liver:

FIGURE 13

Match-up of ATX peptides with putative A2058 protein sequence

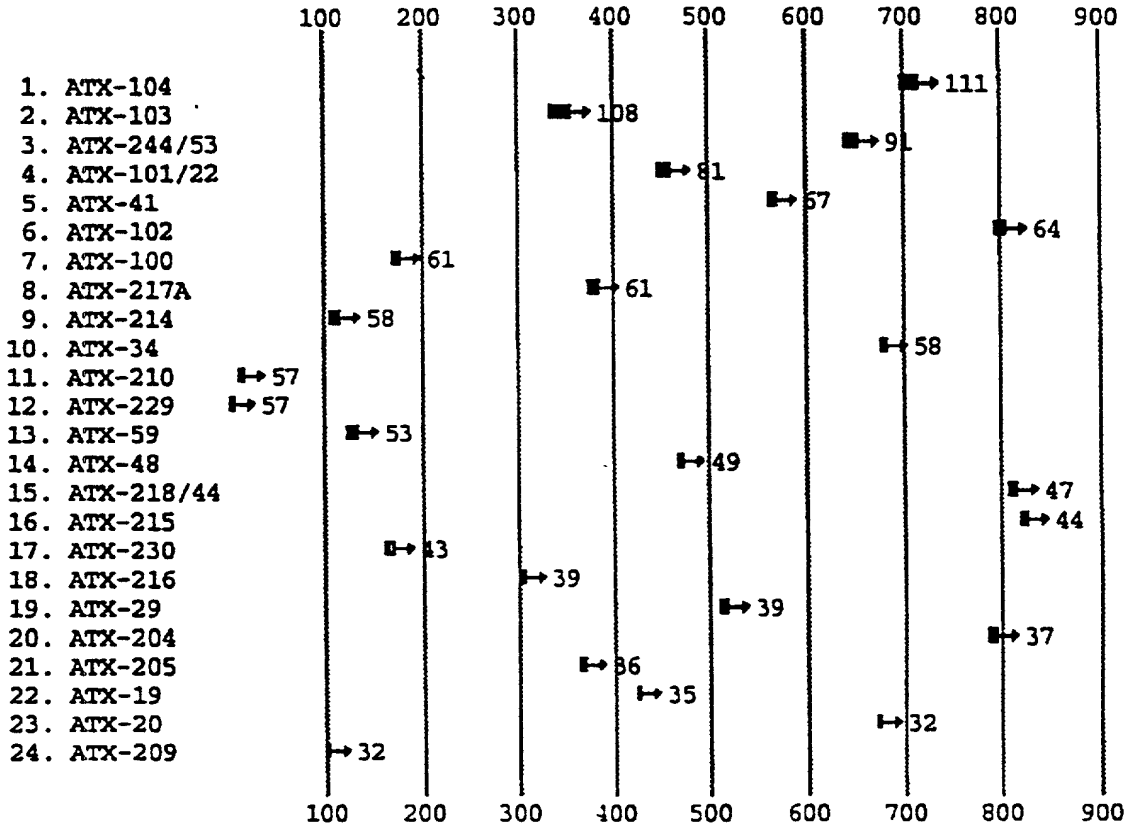


FIGURE 14

Match-up of ATX peptides with putative N-tera 2D1 protein sequence

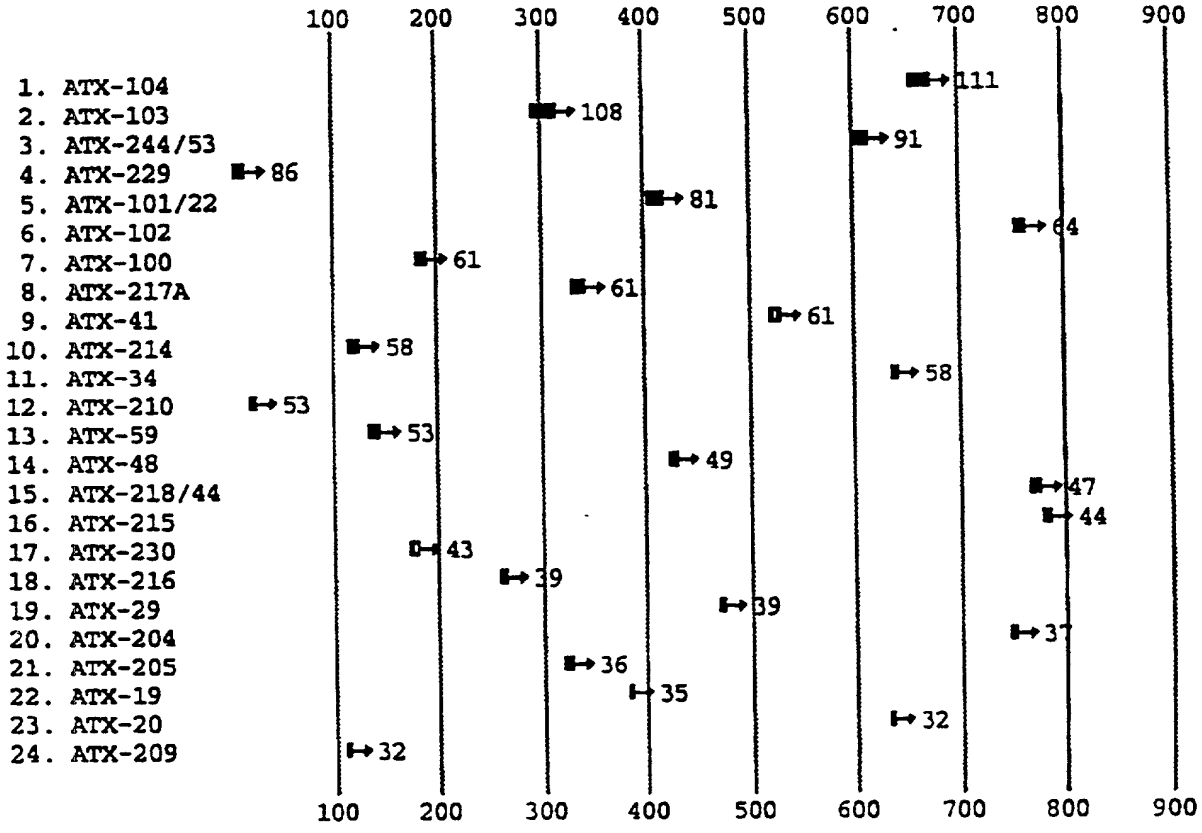


FIGURE 15

FIG. 16

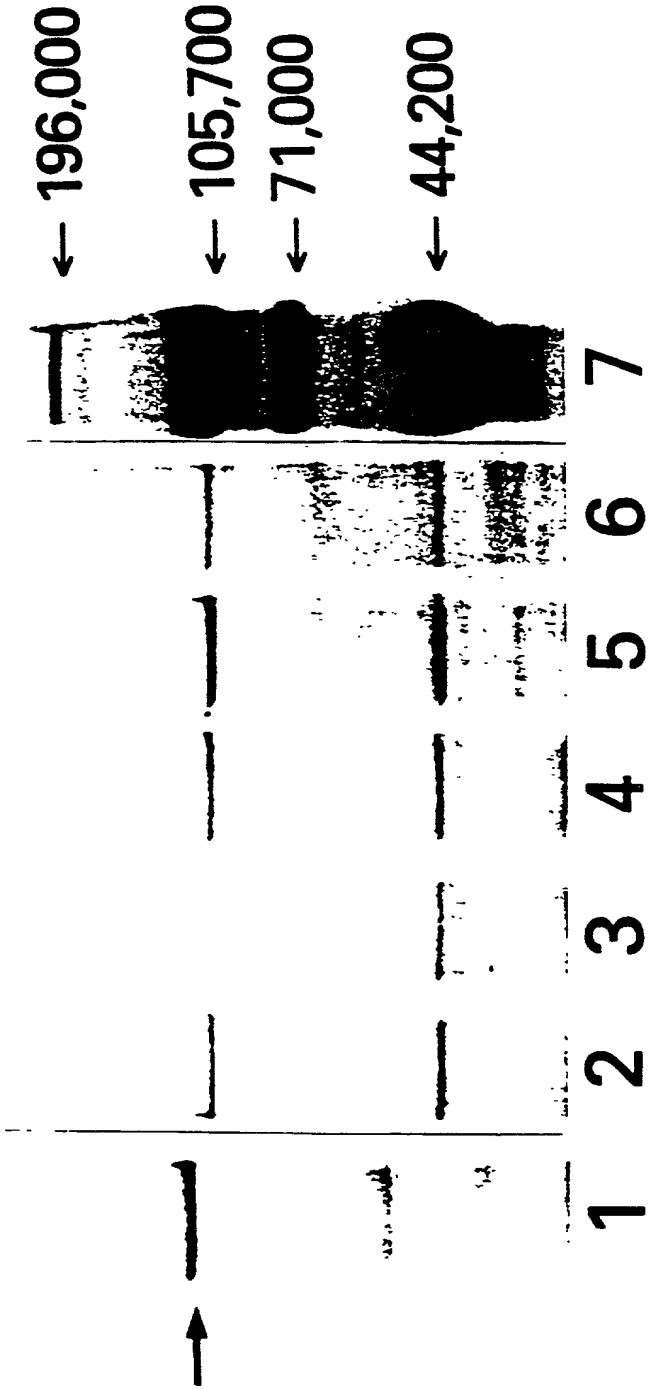
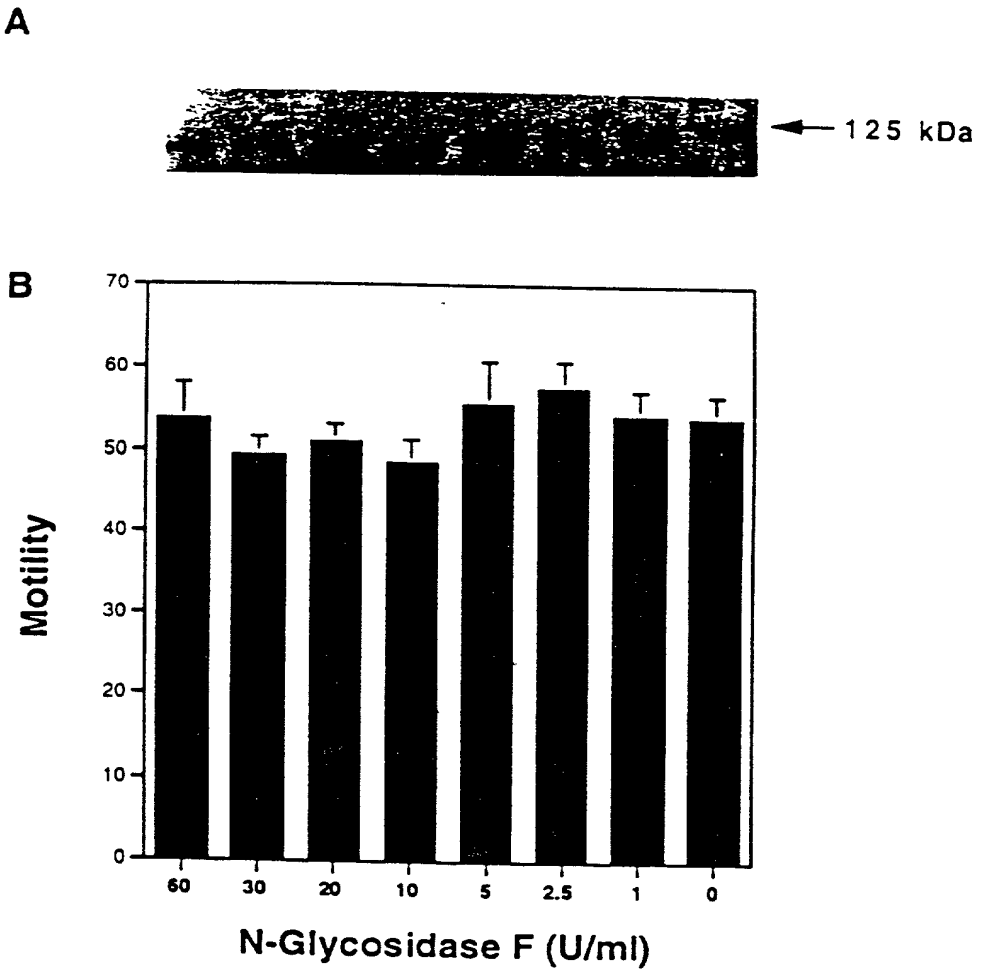


FIG. 17


A
125 kDa
B
Motility
70
60
50
40
30
20
10
0
60
30
20
10
5
2.5
1
0
N-Glycosidase F (U/ml)

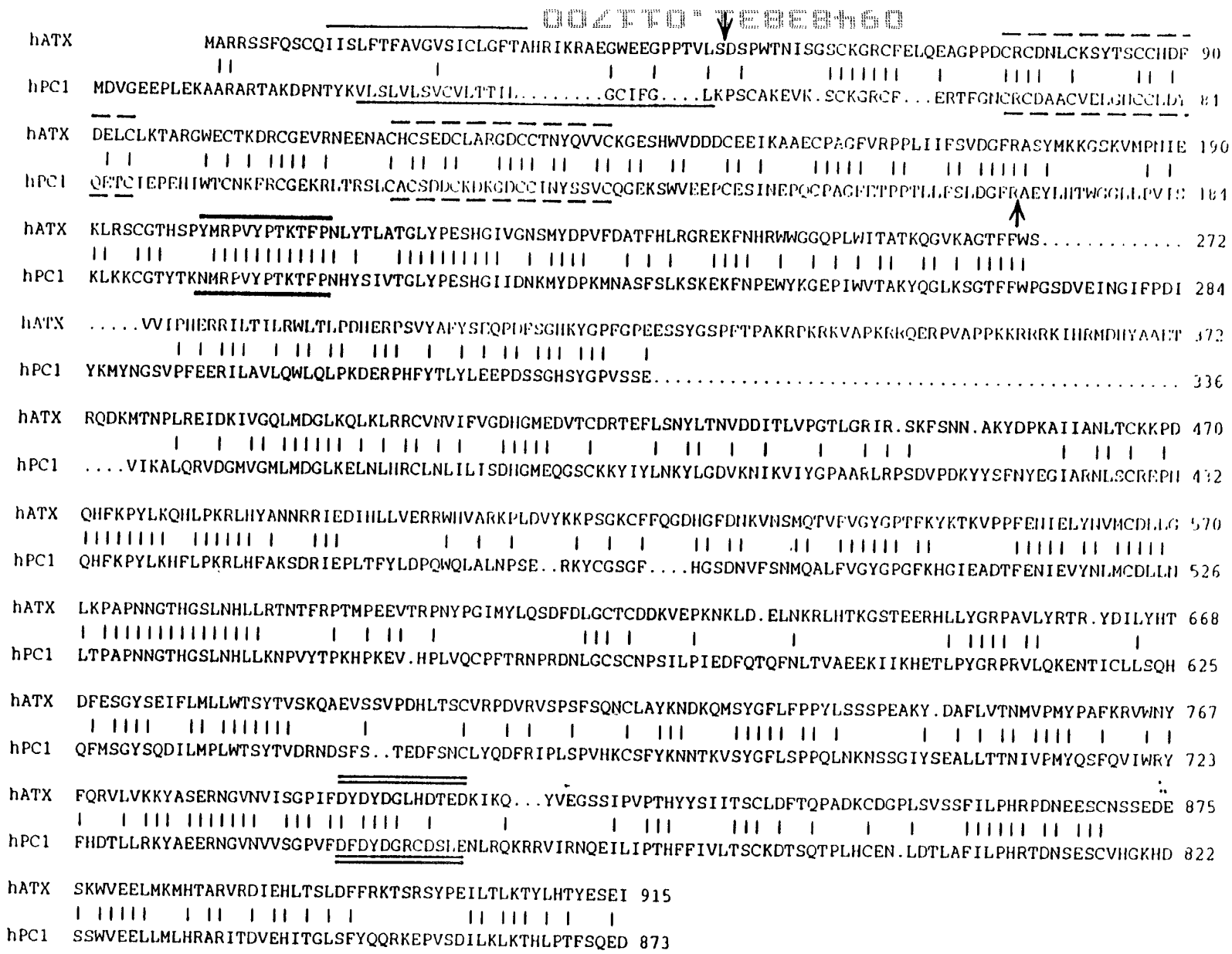
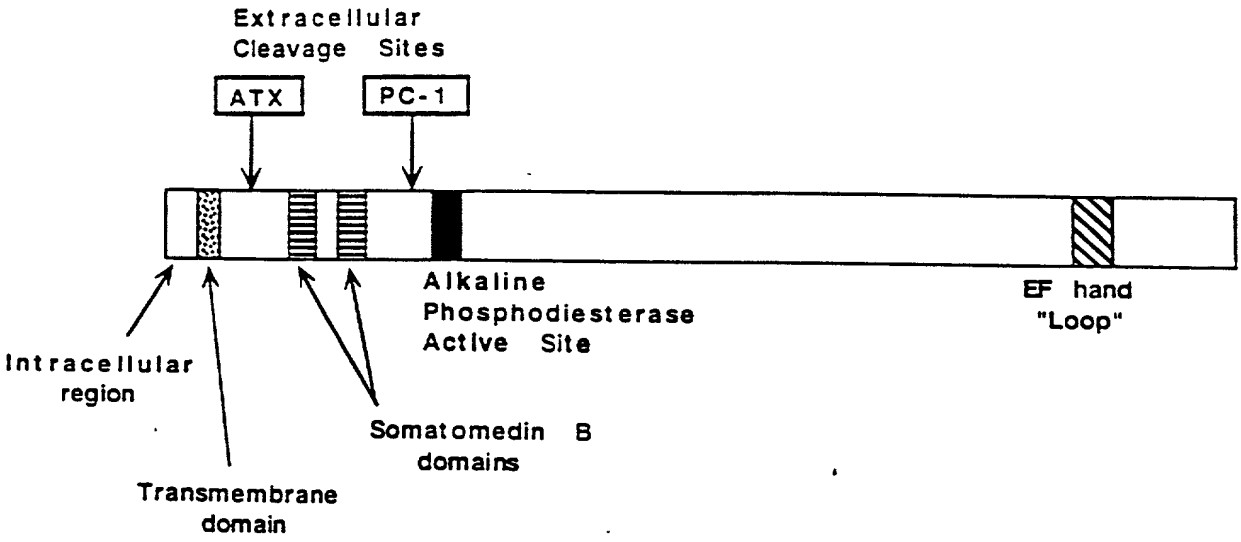


FIG. 18

FIG. 19


Extracellular
Cleavage Sites
ATX
PC-1
Alkaline
Phosphodiesterase
Active Site
EF hand
"Loop"
Intracellular
region
Somatomedin B
domains
Transmembrane
domain

Docket No. 2026-4149US2

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, the information given herein is true, that I believe I am the original, first and sole (if only one name is listed below) or an or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: AUTOTAXIN: MOTILITY STIMULATING PROTEIN USEFUL IN CANCER DIAGNOSIS AND THERAPY

which is described in:
[ ] the attached application or
[ ] PCT International Application No. _____ filed _____
[X] the specification in application Serial No. 08/346,455 filed November 28, 1994
(if applicable) and amended on _____

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information known to me which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56 (a).

I hereby claim foreign priority benefits under Title 35 United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign applications(s) for patent or inventor's certificate or any PCT international applications(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

COUNTRY	APPLICATION	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 USC § 119
			[ ] Yes [ ] No
			[ ] Yes [ ] No
			[ ] Yes [ ] No

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s) or PCT International application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

Application Serial No.	Filing Date	Status: patented, pending, abandoned
07/822,043	January 17, 1992	Pending
08/249,182	May 25, 1994	Pending

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

James C. Haight, Reg. No. 25,588; Gloria Richmond, Reg. No. 30,416; Robert Benson, Reg. No. 33,612; Jack Spiegel, Reg. No. 34,477; Laurence J. Hyman, Reg. No. 35,551; Denise C. Bernstein, Reg. No. 35,787; Susan S. Rucker, Reg. No. 35,762; David R. Sadowski, Reg. No. 32,808 and Ann S. Hobbs, Reg. No. 36,830 and Arthur J. Cohn, Reg. No. 37,800 all of the Office of Technology Transfer, National Institutes of Health, BOX OTT, Bethesda, MD 20892.

I further direct that all correspondence concerning this application be directed to:

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National Institutes of Health
Box OTT
Bethesda, MD 20892
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Fax: (301) 402-0220

EJ604726585US

Docket No. 2026-4149US2

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of first joint inventor: Mary Stracke

Inventor's signature: Mary Stracke Date: 1/31/95

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Post Office Address: 14414 Barkwood Drive, Rockville, Maryland, 20853, U.S.A.

Full Name of second inventor: Lance Liotta

Inventor's signature: Lance Liotta Date: 1-31-95

Country of Citizenship: United States of America

Residence: 9027 Mistwood Drive, Potomac, Maryland, 20854, U.S.A.

Post Office Address: 9027 Mistwood Drive, Potomac, Maryland, 20854, U.S.A.

Full Name of third inventor: Elliott Schiffmann

Inventor's signature: Elliott Schiffmann Date: 1/31/95

Country of Citizenship: United States of America

Residence: 3027 Pickwick Lane, Chevy Chase, Maryland, 20815, U.S.A.

Post Office Address: 3027 Pickwick Lane, Chevy Chase, Maryland, 20815, U.S.A.

Docket No. 2026-4149US2

Full Name of fourth inventor: Jerry Krutzch

Inventor's signature: _____ Date: _____

Country of Citizenship: United States of America

Residence: 9704 De Paul Dirve, Bethesda, Maryland, 20817, U.S.A.

Post Office Address: 9704 De Paul Drive, bethesda, Maryland 20817, U.S.A.

Full Name of first fifth inventor: Jun Murata

Inventor's signature: Jun Murata Date: 2/01/95

Country of Citizenship: Japan

Residence: Dept. of Pathogenic Biochemistry, Research Institute of WAKAN-YAKU, Toyama Medical & Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan

Post Office Address: Dept. of Pathogenic Biochemistry, Research Institute of WAKAN-YAKU, Toyama Medical & Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan

Docket No. 2026-4149US2

Full Name of fourth inventor: Henry Krutzch

Inventor's signature: Henry Krutzch Date: 1/31/95

Country of Citizenship: United States of America

Residence: 9704 De Paul Dirve, Bethesda, Maryland, 20817, U.S.A.

Post Office Address: 9704 De Paul Drive, bethesda, Maryland 20817, U.S.A.

Full Name of first fifth inventor: Jun Murata

Inventor's signature: Date:

Country of Citizenship: Japan

Residence: Dept. of Pathogenic Biochemistry, Research Institute of WAKAN-YAKU, Toyama Medical & Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan

Post Office Address: Dept. of Pathogenic Biochemistry, Research Institute of WAKAN-YAKU, Toyama Medical & Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan

PATENT
Attorney Docket No E-080-94/0
2026-4149US2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of Stracke, et al. Serial Number: 08/346,455 Filed: November 28, 1994	) Art Unit: 1806) Examiner: Loring, S.
For: Autotaxin: Motility Stimulating Protein Useful in Cancer Diagnosis and Therapy	

Associate Power of Attorney

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

The undersigned attorney of record, pursuant to the provisions of 37 CFR §1.33 and § 1.34 M.P.E.P. §402.2, hereby appoints the following as associate attorneys, with full powers to prosecute this application:

Jerome G. Lee, Reg. No. 16,967; John D. Foley, Reg. No. 16,836; John A. Diaz, Reg. No. 19,550; Thomas P. Dowling, Reg. No. 19,221; Eugene Moroz, Reg. No. 25.237; William S. Feiler, Reg. No. 26,728; Israel Blum, Reg. No. 26,710; Mary Morry, Reg. No. 34,398; Maria C. H. Lin, Reg. No. 29,323; Eugene Rzucidlo, Reg. No. 31,900; John C. Vassil, Reg. No. 19,098; Leslie A. Serunian, Reg. No. 35,353; Kathryn M. Brown, Reg. No. 34,556; Dorothy R. Auth, Reg. No. 36,434 and Richard W. Bork, Reg. No. 36,459;

all of the law firm of MORGAN & FINNEGAN, 345 Park Avenue, New York, NY 10154.

Dated: March 14, 1996

Respectfully submitted,

Susan S. Rucker
Attorney for Applicant(s)
Reg. No. 35,762

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National Institutes of Health
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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: UNITED STATES OF AMERICA; DEPT. OF HEALTH AND HUMAN SERVICES

(ii) TITLE OF INVENTION: MOTILITY STIMULATING PROTEIN USEFUL IN CANCER DIAGNOSIS AND THERAPY

(iii) NUMBER OF SEQUENCES: 69

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: MORGAN & FINNEGAN
(B) STREET: 345 PARK AVENUE
(C) CITY: NEW YORK
(D) STATE: NEW YORK
(E) COUNTRY: U.S.A.
(F) ZIP: 10154

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy Disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: WordPerfect 5.1

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA
(A) APPLICATION NUMBER: 08/346,455
(B) FILING DATE: 28-NOV-1994

(vii) PRIOR APPLICATION DATA
(A) APPLICATION NUMBER: 08/249,182
(B) FILING DATE: 25-MAY-1994

(vii) PRIOR APPLICATION DATA
(A) APPLICATION NUMBER: 07/822,043
(B) FILING DATE: 17-JAN-1992

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: DOROTHY R. AUTH
(B) REGISTRATION NUMBER: 36,434
(C) DOCKET NUMBER: 2026-4149US3

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (212) 758-4800
(B) TELEFAX: (212) 751-6849

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Trp His Val Ala Arg
1 5

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Pro Leu Asp Val Tyr Lys
1 5

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Tyr Pro Ala Phe Lys
1 5

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Gln Ala Glu Val Ser
1 5

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Pro Glu Glu Val Thr Arg Pro Asn Tyr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Tyr Asp Val Pro Trp Asn Glu Thr Ile
1 5

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Val Pro Pro Phe Glu Asn Ile Glu Leu Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Gly Gly Gln Pro Leu Trp Ile Thr Ala Thr Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Val Asn Ser Met Gln Thr Val Phe Val Gly Tyr Gly
1 5 10

Pro Thr Phe Lys
15

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Asp Ile Glu His Leu Thr Ser Leu Asp Phe Phe Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Thr Glu Phe Leu Ser Asn Tyr Leu Thr Asn Val Asp
1 5 10

Asp Ile Thr Leu Val Pro Glu Thr Leu Gly Arg
15 20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTTGGCAGCN ACRTGCCA 18

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TGGCAYGTNG CTGCCAAC 18

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTTGAAGGCA GGGTA 15

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TAYCCTGCNT TYAAG 15

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGTNACYTCY TCAGG 15

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CCTGARGARG TNACC 15

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

NGTNGCRTCR AATGGCACRT C 21

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GAYGTGCCAT TYGAYGCNAC N 21

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GTTDATRTTS TCRAATGGGG G 21

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CCCCCATTTG AGAACATCAA C 21

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CTTNGTNGCN GTDATCCANA RGGGYTGGCC GCC 33

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GGCGGCCARC CCYTNTGGAT HACNGCNACN AAG 33

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CTTRAAGGTG GGGCCRTAGC CCACRAAGAC TGTYTGCAT 39

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

ATGCARACAG TCTTYGTGGG CTAYGGCCCC ACCTTYAAR 39

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Gln Tyr Leu His Gln Tyr Gly Ser Ser
1 5

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Val Leu Asn Tyr Phe
1 5

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Tyr Leu Asn Ala Thr
1 5

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

His Leu Leu Tyr Gly Arg Pro Ala Val Leu Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ser Tyr Pro Glu Ile Leu Thr Pro Ala Asp Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Xaa Tyr Gly Phe Leu Phe Pro Pro Tyr Leu Ser Ser
1 5 10
Ser Pro

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
Thr Phe Pro Asn Leu Tyr Thr Phe Ala Thr Gly Leu
  1               5                  10
Tyr
```

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
Val Asn Val Ile Ser Gly Pro Ile Asp Asp Tyr Asp
  1               5                  10
Tyr Asp Gly Leu His Asp Thr Glu Asp Lys
         15                  20
```

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 829
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: No

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Human
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Melanoma
(H) CELL LINE: A2058
(I) ORGANELLE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION: Putative protein sequence of A2058 Autotaxin

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```
Cys His Asp Phe Asp Glu Leu Cys Leu Lys Thr Ala
 1               5                  10
Arg Gly Trp Glu Cys Thr Lys Asp Arg Cys Gly Glu
        15                  20
Val Arg Asn Glu Glu Asn Ala Cys His Cys Ser Glu
25                  30                  35
Asp Cys Leu Ala Arg Gly Asp Cys Cys Thr Asn Tyr
            40                  45
Gln Val Val Cys Lys Gly Glu Ser His Trp Val Asp
    50                  55                      60
Asp Asp Cys Glu Glu Ile Lys Ala Ala Glu Cys Pro
                65                  70
Ala Gly Phe Val Arg Pro Pro Leu Ile Ile Phe Ser
        75                  80
Val Asp Gly Phe Arg Ala Ser Tyr Met Lys Lys Gly
85                  90                  95
Ser Lys Val Met Pro Asn Ile Glu Lys Leu Arg Ser
            100                 105
Cys Gly Thr His Ser Pro Tyr Met Arg Pro Val Tyr
    110                 115                     120
Pro Thr Lys Thr Phe Pro Asn Leu Tyr Thr Leu Ala
                125                 130
Thr Gly Leu Tyr Pro Glu Ser His Gly Ile Val Gly
        135                 140
Asn Ser Met Tyr Asp Pro Val Phe Asp Ala Thr Phe
145                 150                 155
His Leu Arg Gly Arg Glu Lys Phe Asn His Arg Trp
            160                 165
Trp Gly Gly Gln Pro Leu Trp Ile Thr Ala Thr Lys
    170                 175                     180
Gln Gly Val Lys Ala Gly Thr Phe Phe Trp Ser Val
                185                 190
Val Ile Pro His Glu Arg Arg Ile Leu Thr Ile Leu
        195                 200
Arg Trp Leu Thr Leu Pro Asp His Glu Arg Pro Ser
205                 210                 215
Val Tyr Ala Phe Tyr Ser Glu Gln Pro Asp Phe Ser
            220                 225
Gly His Lys Tyr Gly Pro Phe Gly Pro Glu Glu Ser
    230                 235                     240
Ser Tyr Gly Ser Pro Phe Thr Pro Ala Lys Arg Pro
                245                 250
Lys Arg Lys Val Ala Pro Lys Arg Arg Gln Glu Arg
        255                 260
Pro Val Ala Pro Pro Lys Lys Arg Arg Arg Lys Ile
265                 270                 275
His Arg Met Asp His Tyr Ala Ala Glu Thr Arg Gln
            280                 285
```

```
Asp Lys Met Thr Asn Pro Leu Arg Glu Ile Asp Lys
    290                 295                 300
Ile Val Gly Gln Leu Met Asp Gly Leu Lys Gln Leu
                305                 310
Lys Leu Arg Arg Cys Val Asn Val Ile Phe Val Gly
            315                 320
Asp His Gly Met Glu Asp Val Thr Cys Asp Arg Thr
325                 330                 335
Glu Phe Leu Ser Asn Tyr Leu Thr Asn Val Asp Asp
                340                 345
Ile Thr Leu Val Pro Gly Thr Leu Gly Arg Ile Arg
    350                 355                 360
Ser Lys Phe Ser Asn Asn Ala Lys Tyr Asp Pro Lys
                365                 370
Ala Ile Ile Ala Asn Leu Thr Cys Lys Lys Pro Asp
            375                 380
Gln His Phe Lys Pro Tyr Leu Lys Gln His Leu Pro
385                 390                 395
Lys Arg Leu His Tyr Ala Asn Asn Arg Arg Ile Glu
                400                 405
Asp Ile His Leu Leu Val Glu Arg Arg Trp His Val
    410                 415                 420
Ala Arg Lys Pro Leu Asp Val Tyr Lys Lys Pro Ser
                425                 430
Gly Lys Cys Phe Phe Gln Gly Asp His Gly Phe Asp
            435                 440
Asn Lys Val Asn Ser Met Gln Thr Val Phe Val Gly
445                 450                 455
Tyr Gly Pro Thr Phe Lys Tyr Lys Thr Lys Val Pro
                460                 465
Pro Phe Glu Asn Ile Glu Leu Tyr Asn Val Met Cys
    470                 475                 480
Asp Leu Leu Gly Leu Lys Pro Ala Pro Asn Asn Gly
                485                 490
Thr His Gly Ser Leu Asn His Leu Leu Arg Thr Asn
            495                 500
Thr Phe Arg Pro Thr Met Pro Glu Glu Val Thr Arg
505                 510                 515
Pro Asn Tyr Pro Gly Ile Met Tyr Leu Gln Ser Asp
                520                 525
Asp Asp Leu Gly Cys Thr Cys Asp Asp Lys Val Glu
    530                 535                 540
Pro Lys Asn Lys Leu Asp Glu Leu Asn Lys Arg Leu
                545                 550
His Thr Lys Gly Ser Thr Glu Glu Arg His Leu Leu
            555                 560
Tyr Gly Arg Pro Ala Val Leu Tyr Arg Thr Arg Tyr
565                 570                 575
Asp Ile Leu Tyr His Thr Asp Phe Glu Ser Gly Tyr
                580                 585
Ser Glu Ile Phe Leu Met Leu Leu Trp Thr Ser Tyr
    590                 595                 600
Thr Val Ser Lys Gln Ala Glu Val Ser Ser Val Pro
                605                 610
```

```
Asp His Leu Thr Ser Cys Val Arg Pro Asp Val Arg
          615                 620
Val Ser Pro Ser Phe Ser Gln Asn Cys Leu Ala Tyr
625                 630                 635
Lys Asn Asp Lys Gln Met Ser Tyr Gly Phe Leu Phe
               640                 645
Pro Pro Tyr Leu Ser Ser Ser Pro Glu Ala Lys Tyr
     650                 655                 660
Asp Ala Phe Leu Val Thr Asn Met Val Pro Met Tyr
                    665                 670
Pro Ala Phe Lys Arg Val Trp Asn Tyr Phe Gln Arg
          675                 680
Val Leu Val Lys Lys Tyr Ala Ser Glu Arg Asn Gly
685                 690                 695
Val Asn Val Ile Ser Gly Pro Ile Phe Asp Tyr Asp
               700                 705
Tyr Asp Gly Leu His Asp Thr Glu Asp Lys Ile Lys
     710                 715                 720
Gln Tyr Val Glu Gly Ser Ser Ile Pro Val Pro Thr
                    725                 730
His Tyr Tyr Ser Ile Ile Thr Ser Cys Leu Asp Phe
          735                 740
Thr Gln Pro Ala Asp Lys Cys Asp Gly Pro Leu Ser
745                 750                 755
Val Ser Ser Phe Ile Leu Pro His Arg Pro Asp Asn
               760                 765
Glu Glu Ser Cys Asn Ser Ser Glu Asp Glu Ser Lys
     770                 775                 780
Trp Val Glu Glu Leu Met Lys Met His Thr Ala Arg
                    785                 790
Val Arg Asp Ile Glu His Leu Thr Ser Leu Asp Phe
          795                 800
Phe Arg Lys Thr Ser Arg Ser Tyr Pro Glu Ile Leu
805                 810                 815
Thr Leu Lys Thr Tyr Leu His Thr Tyr Glu Ser Glu
               820                 825
Ile
```

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2946
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Human
(B) STRAIN:

(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Melanoma
(H) CELL LINE: A2058
(I) ORGANELLE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: Partial DNA Sequence of A2058 Autotaxin

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GCTGCCATGA CTTTGATGAG CTGTGTTTGA AGACAGCCCG 40
TGGCTGGGAG TGTACTAAGG ACAGATGTGG AGAAGTCAGA 80
AATGAAGAAA ATGCCTGTCA CTGCTCAGAG GACTGCTTGG 120
CCAGGGGAGA CTGCTGTACC AATTACCAAG TGGTTTGCAA 160
AGGAGAGTCG CATTGGGTTG ATGATGACTG TGAGGAAATA 200
AAGGCCGCAG AATGCCCTGC AGGGTTTGTT CGCCCTCCAT 240
TAATCATCTT CTCCGTGGAT GGCTTCCGTG CATCATACAT 280
GAAGAAAGGC AGCAAAGTCA TGCCTAATAT TGAAAAACTA 320
AGGTCTTGTG GCACACACTC TCCCTACATG AGGCCGGTGT 360
ACCCAACTAA AACCTTTCCT AACTTATACA CTTTGGCCAC 400
TGGGCTATAT CCAGAATCAC ATGGAATTGT TGGCAATTCA 440
ATGTATGATC CTGTATTTGA TGCCACTTTT CATCTGCGAG 480
GGCGAGAGAA ATTTAATCAT AGATGGTGGG GAGGTCAACC 520
GCTATGGATT ACAGCCACCA AGCAAGGGGT GAAAGCTGGA 560
ACATTCTTTT GGTCTGTTGT CATCCCTCAC GAGCGGAGAA 600
TATTAACCAT ATTGCGGTGG CTCACCCTGC CAGATCATGA 640
GAGGCCTTCG GTCTATGCCT TCTATTCTGA GCAACCTGAT 680
TTCTCTGGAC ACAAATATGG CCCTTTCGGC CCTGAGGAGA 720
GTAGTTATGG CTCACCTTTT ACTCCGGCTA AGAGACCTAA 760
GAGGAAAGTT GCCCCTAAGA GGAGACAGGA AAGACCAGTT 800
GCTCCTCCAA AGAAAAGAAG AAGAAAAATA CATAGGATGG 840
ATCATTATGC TGCGGAAACT CGTCAGGACA AAATGACAAA 880
TCCTCTGAGG GAAATCGACA AAATTGTGGG GCAATTAATG 920
GATGGACTGA AACAACTAAA ACTGCGTCGG TGTGTCAACG 960
TCATCTTTGT CGGAGACCAT GGAATGGAAG ATGTCACATG 1000
TGATAGAACT GAGTTCTTGA GTAATTACCT AACTAATGTG 1040
GATGATATTA CTTTAGTGCC TGGAACTCTA GGAAGAATTC 1080
GATCCAAATT TAGCAACAAT GCTAAATATG ACCCCAAAGC 1120
CATTATTGCC AATCTCACGT GTAAAAAACC AGATCAGCAC 1160
TTTAAGCCTT ACTTGAAACA GCACCTTCCC AAACGTTTGC 1200
ACTATGCCAA CAACAGAAGA ATTGAGGATA TCCATTTATT 1240
GGTGGAACGC AGATGGCATG TTGCAAGGAA ACCTTTGGAT 1280
GTTTATAAGA AACCATCAGG AAAATGCTTT TTCCAGGGAG 1320
ACCACGGATT TGATAACAAG GTCAACAGCA TGCAGACTGT 1360
TTTTGTAGGT TATGGCCCAA CATTTAAGTA CAAGACTAAA 1400
GTGCCTCCAT TTGAAAACAT TGAACTTTAC AATGTTATGT 1440
GTGATCTCCT GGGATTGAAG CCAGCTCCTA ATAATGGGAC 1480

CCATGGAAGT TTGAATCATC TCCTGCGCAC TAATACCTTC 1520
AGGCCAACCA TGCCAGAGGA AGTTACCAGA CCCAATTATC 1560
CAGGGATTAT GTACCTTCAG TCTGATTTTG ACCTGGGCTG 1600
CACTTGTGAT GATAAGGTAG AGCCAAAGAA CAAGTTGGAT 1640
GAACTCAACA AACGGCTTCA TACAAAAGGG TCTACAGAAG 1680
AGAGACACCT CCTCTATGGG CGACCTGCAG TGCTTTATCG 1720
GACTAGATAT GATATCTTAT ATCACACTGA CTTTGAAAGT 1760
GGTTATAGTG AAATATTCCT AATGCTACTC TGGACATCAT 1800
ATACTGTTTC CAAACAGGCT GAGGTTTCCA GCGTTCCTGA 1840
CCATCTGACC AGTTGCGTCC GGCCTGATGT CCGTGTTTCT 1880
CCGAGTTTCA GTCAGAACTG TTTGGCCTAC AAAAATGATA 1920
AGCAGATGTC CTACGGATTC CTCTTTCCTC CTTATCTGAG 1960
CTCTTCACCA GAGGCTAAAT ATGATGCATT CCTTGTAACC 2000
AATATGGTTC CAATGTATCC TGCTTTCAAA CGGGTCTGGA 2040
ATTATTTCCA AAGGGTATTG GTGAAGAAAT ATGCTTCGGA 2080
AAGAAATGGA GTTAACGTGA TAAGTGGACC AATCTTCGAC 2120
TATGACTATG ATGGCTTACA TGACACAGAA GACAAAATAA 2160
AACAGTACGT GGAAGGCAGT TCCATTCCTG TTCCAACTCA 2200
CTACTACAGC ATCATCACCA GCTGTCTGGA TTTCACTCAG 2240
CCTGCCGACA AGTGTGACGG CCCTCTCTCT GTGTCCTCCT 2280
TCATCCTGCC TCACCGGCCT GACAAAGAGG AGAGCTGCAA 2320
TAGCTCAGAG GACGAATCAA AATGGGTAGA AGAACTCATG 2360
AAGATGCACA CAGCTAGGGT GCGTGACATT GAACATCTCA 2400
CCAGCCTGGA CTTCTTCCGA AAGACCAGCC GCAGCTACCC 2440
AGAAATCCTG ACACTCAAGA CATACCTGCA TACATATGAG 2480
AGCGAGATTT AACTTTCTGA GCATCTGCAG TACAGTCTTA 2520
TCAACTGGTT GTATATTTTT ATATTGTTTT TGTATTTATT 2560
AATTTGAAAC CAGGACATTA AAAATGTTAG TATTTTAATC 2600
CTGTACCAAA TCTGACATAT TATGCCTGAA TGACTCCACT 2640
GTTTTTCTCT AATGCTTGAT TTAGGTAGCC TTGTGTTCTG 2680
AGTAGAGCTT GTAATAAATA CTGCAGCTTG AGAAAAAGTG 2720
GAAGCTTCTA AATGGTGCTG CAGATTTGAT ATTTGCATTG 2760
AGGAAATATT AATTTTCCAA TGCACAGTTG CCACATTTAG 2800
TCCTGTACTG TATGGAAACA CTGATTTTGT AAAGTTGCCT 2840
TTATTTGCTG TTAACTGTTA ACTATGACAG ATATATTTAA 2880
GCCTTATAAA CCAATCTTAA ACATAATAAA TCACACATTC 2920
AGTTTTAAAA AAAAAAAAAA AAAAAA 2946

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 788
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: No

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Human
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:

(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: teratocarcinoma
(H) CELL LINE: N-tera 2D1
(I) ORGANELLE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: N-tera 2D1 putative ATX protein sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```
Cys Asp Asn Leu Cys Lys Ser Tyr Thr Ser Cys Cys
 1               5                  10
His Asp Phe Asp Glu Leu Cys Leu Lys Thr Ala Arg
            15                  20
Ala Trp Glu Cys Thr Lys Asp Arg Cys Gly Glu Val
 25                  30                  35
Arg Asn Glu Glu Asn Ala Cys His Cys Ser Glu Asp
            40                  45
Cys Leu Ala Arg Gly Asp Cys Cys Thr Asn Tyr Gln
     50                  55                  60
Val Val Cys Lys Gly Glu Ser His Trp Val Asp Asp
                65                  70
Asp Cys Glu Glu Ile Lys Ala Ala Glu Cys Leu Gln
            75                  80
Val Asp Ser Pro Ser Ile Asn His Leu Leu Arg Gly
 85                  90                  95
Trp Leu Pro Met Thr Ser Tyr Met Lys Lys Gly Ser
            100                 105
Lys Val Met Pro Asn Ile Glu Lys Leu Arg Ser Cys
    110                 115                 120
Gly Thr His Ser Pro Tyr Met Arg Pro Val Tyr Pro
                125                 130
Thr Lys Thr Phe Pro Asn Leu Tyr Thr Leu Ala Thr
            135                 140
Gly Leu Tyr Pro Glu Ser His Gly Ile Val Gly Asn
145                 150                 155
Ser Met Tyr Asp Pro Val Phe Asp Ala Thr Phe His
                160                 165
Leu Arg Gly Arg Glu Lys Phe Asn His Arg Trp Trp
    170                 175                 180
Ala Gly Gln Pro Leu Trp Ile Thr Ala Thr Lys Gln
                185                 190
Arg Gly Glu Ser Trp Asn Ile Leu Leu Val Cys Cys
            195                 200
His Pro Ser Arg Ala Glu Ile Leu Thr Ile Leu Gln
205                 210                 215
Trp Leu Thr Leu Pro Asp His Glu Arg Pro Ser Val
            220                 225
```

```
Tyr Ala Phe Tyr Ser Glu Gln Pro Asp Phe Ser Gly
    230                 235                 240
His Lys His Met Pro Phe Gly Pro Glu Met Pro Asn
                245                 250
Pro Leu Arg Glu Met His Lys Ile Val Gly Gln Leu
        255                 260
Met Asp Gly Leu Lys Gln Leu Lys Leu His Arg Cys
265                 270                 275
Val Asn Val Ile Phe Val Glu Thr Met Asp Gly Arg
            280                 285
Cys His Met Tyr Arg Thr Glu Phe Leu Ser Asn Tyr
    290                 295                 300
Leu Thr Asn Val Asp Asp Ile Thr Leu Val Pro Gly
                305                 310
Thr Leu Gly Arg Ile Arg Ser Lys Phe Ser Asn Asn
        315                 320
Ala Lys Tyr Asp Pro Lys Ala Ile Ile Ala Asn Leu
325                 330                 335
Thr Cys Lys Lys Pro Asp Gln His Phe Lys Pro Tyr
            340                 345
Leu Lys Gln His Leu Pro Lys Arg Leu His Tyr Ala
    350                 355                 360
Asn Asn Arg Arg Ile Glu Asp Ile His Leu Leu Val
                365                 370
Glu Arg Arg Trp His Val Ala Arg Lys Pro Leu Asp
        375                 380
Val Tyr Lys Lys Pro Ser Gly Asn Ala Phe Ser Arg
385                 390                 395
Glu Thr Thr Ala Phe Asp Asn Lys Val Asn Ser Met
            400                 405
Gln Thr Val Phe Val Gly Tyr Gly Pro Thr Phe Lys
    410                 415                 420
Tyr Lys Thr Lys Val Pro Pro Phe Glu Asn Ile Glu
                425                 430
Leu Tyr Asn Val Met Cys Asp Leu Leu Gly Leu Lys
        435                 440
Pro Ala Pro Asn Asn Gly Thr His Phe Ser Leu Asn
445                 450                 455
His Leu Leu Arg Thr Asn Thr Phe Arg Pro Thr Met
            460                 465
Pro Glu Glu Val Thr Arg Pro Asn Tyr Pro Gly Ile
    470                 475                 480
Met Tyr Leu Gln Ser Asp Phe Asp Leu Gly Cys Thr
                485                 490
Cys Asp Asp Lys Val Glu Pro Lys Asn Lys Leu Asp
        495                 500
Glu Leu Asn Lys Arg Leu His Thr Lys Gly Ser Thr
505                 510                 515
Glu Glu Arg His Leu Leu Tyr Gly Asp Arg Pro Ala
            520                 525
Val Leu Tyr Arg Thr Arg Tyr Asp Ile Leu Tyr His
    530                 535                 540
Thr Asp Phe Glu Ser Gly Tyr Ser Glu Ile Phe Leu
                545                 550
```

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Met Pro Leu Trp Thr Ser Tyr Thr Val Ser Lys Gln
        555                 560
Ala Glu Val Ser Ser Val Pro Asp His Leu Thr Ser
565                 570                 575
Cys Val Arg Pro Asp Val Arg Val Ser Pro Ser Phe
            580                 585
Ser Gln Asn Cys Leu Ala Tyr Lys Asn Asp Lys Gln
    590                 595                 600
Met Ser Tyr Gly Gly Leu Gly Pro Pro Tyr Leu Ser
                605                 610
Ser Ser Pro Glu Ala Lys Tyr Asp Ala Phe Leu Val
        615                 620
Thr Asn Met Val Pro Met Tyr Pro Ala Phe Lys Arg
625                 630                 635
Val Trp Asn Tyr Phe Gln Arg Val Leu Val Lys Lys
            640                 645
Tyr Ala Ser Glu Arg Asn Gly Val Asn Val Ile Ser
    650                 655                 660
Gly Pro Ile Phe Asp Tyr Asp Tyr Asp Gly Leu His
                665                 670
Asp Thr Glu Asp Lys Ile Lys Gln Tyr Val Glu Gly
        675                 680
Ser Ser Ile Pro Val Pro Thr His Tyr Tyr Ser Ile
685                 690                 695
Ile Thr Ser Cys Leu Asp Phe Thr Gln Pro Ala Asp
            700                 705
Lys Cys Asp Gly Pro Leu Ser Val Ser Ser Phe Ile
    710                 715                 720
Leu Pro His Arg Pro Asp Asn Glu Glu Ser Cys Asn
                725                 730
Ser Ser Glu Asp Glu Ser Lys Trp Val Glu Glu Leu
        735                 740
Met Lys Met His Thr Ala Arg Val Arg Asp Ile Glu
745                 750                 755
His Leu Thr Ser Leu Asp Phe Phe Arg Lys Thr Ser
            760                 765
Arg Ser Tyr Pro Glu Ile Leu Thr Leu Lys Thr Tyr
    770                 775                 780
Leu His Thr Tyr Glu Ser Glu Ile
                785
```

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2712
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Human
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: teratocarcinoma
(H) CELL LINE: N-tera 2D1
(I) ORGANELLE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: N-tera 2D1 ATX DNA sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

TGTGACAACT TGTGTAAGAG CTATACCAGT TGCTGCCATG 40
ACTTTGATGA GCTGTGTTTG AAGACAGCCC GTGCGTGGGA 80
GTGTACTAAG GACAGATGTG GGGAAGTCAG AAATGAAGAA 120
AATGCCTGTC ACTGCTCAGA GGACTGCTTG GCCAGGGGAG 160
ACTGCTGTAA CAATTACCAA GTGGTTTGCA AAGGAGAGTC 200
GCATTGGGTT GATGATGACT GTGAGGAAAT AAAGGCCGCA 240
GAATGCCTGC AGGTTTGTTC GCCCTCCATT AATCATCTTC 280
TCCGTGGATG GCTTCCGATG ACATCATACA TGAAGAAAGG 320
CAGCAAAGTC ATGCCTAATA TTGAAAAACT AAGGTCTTGT 360
GGCACACACT CTCCCTACAT GAGGCCGGTG TACCCAACTA 400
AAACCTTTCC TAACTTATAC ACTTTGGCCA CTGGGCTATA 440
TCCAGAATCA CATGGAATTG TTGGCAATTC AATGTATGAT 480
CCTGTATTTG ATGCCACTTT TCATCTGCGA GGGCGAGAGA 520
AATTTAATCA TAGATGGTGG GGAGGTCAAC CGCTATGGAT 560
TACAGCCACC AAGCAAAGGG GTGAAAGCTG GAACATTCTT 600
TTGGTCTGTT GTCATCCCTC ACGAGCGGAG ATATTAACCA 640
TATTGCAGTG GCTCACCCTG CCAGATCATG AGAGGCCTTC 680
GGTCTATGCC TTCTATTCTG AGCAACCTGA TTTCTCTGGA 720
CACAAACATA TGCCTTTCGG CCCTGAGATG ACAAATCCTC 760
TGAGGGAAAT GCACAAAATT GTGGGGCAAT TAATGGATGG 800
ACTGAAACAA CTAAAACTGC ATCGGTGTGT CAACGTCATC 840
TTTGTCGAGA CCATGGATGG AAGATGTCAC ATGTATAGAA 880
CTGAGTTCTT GAGTAATTAC CTAACTAATG TGGATGATAT 920
TACTTTAGTG CCTGGAACTC TAGGAAGAAT TCGATCCAAA 960
TTTAGCAACA ATGCTAAATA TCACCCCAAA GCCATTATTG 1000
CCAATCTCAC GTGTAAAAAA CCAGATCAGC ACTTTAAGCC 1040
TTACTTGAAA CAGCACCTTC CCAAACGTTT GCACTATGCC 1080
AACAACAGAA GAATTGAGGA TATCCATTTA TTGGTGGAAC 1120
GCAGATGGCA TGTTGCAAGG AAACCTTTGG ATGTTTATAA 1160
GAAACCATCA GGAAATGCTT TTTCCAGGGA GACCACGGCA 1200
TTTGATAACA AGGTCAACAG CATGCAGACT GTTTTTGTAG 1240
GTTATGGCCC AACATTTAAG TACAAGACTA AAGTDCCTCC 1280
ATTTGAAAAC ATTGAACTTT AAAATGTTAT GTGTGATCTC 1320

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CTGGGATTGA AGCCAGCTCC TAATAATGGG ACCCATGGAA          1360
GTTTGAATCA TCTCCTGCGC ACTAATACCT TCAGGCCAAC          1400
CATGCCAGAG GAAGTTACCA GACCCTATTA TCCAGGGATT          1440
ATGTACCTTC AGTCTGATTT TGACCTGGGC TGCACTTGTG          1480
ATGATAAGGT AGAGCCAAAG AACAAGTTGG ATGAACTCAA          1520
CAAACGGCTT CATACAAAAG GGTCTACAGA AGAGAGACAC          1560
CTCCTCTATG GGGATCGACC TGCAGTGCTT TATCGGACTA          1600
GATATGATAT CTTATATCAC ACTGACTTTG AAAGTGGTTA          1640
TAGTGAAATA TTCCTAATGC CACTCTGGAC ATCATATACT          1680
GTTTCCAAAC AGGCTGAGGT TTCCAGCGTT CCTGACCATC          1720
TGACCAGTTG CGTCCGGCCT GATGTCCGTG TTTCTCCGAG          1760
TTTCAGTCAG AACTGTTTGG CCTACAAAAA TGATAAGCAG          1800
ATGTCCTACG GATTCCTCTT TCCTCCTTAT CTGAGCTCTT          1840
CACCAGAGGC TAAATATGAT GCATTCCTTG TAACCAATAT          1880
GGTTCCAATG TATCCTGCTT TCAAACGGGT CTGGAATTAT          1920
TTCCAAAGGG TATTGGTGAA GAAATATGCT TCGGAAAGAA          1960
ATGGAGTTAA CGTGATAAGT GGACCAATCT TCGACTATGA          2000
CTATGATGGC TTACATGACA CAGAAGACAA AATAAAACAG          2040
TACGTGGAAG GCAGTTCCAT TCCTGTTCCA ACTCACTACT          2080
ACAGCATCAT CACCAGCTGT CTGGATTTCA CTCAGCCTGC          2120
CGACAAGTGT GACGGCCCTC TCTCTGTGTC CTCCTTCATC          2160
CTGCCTCACC GGCCTGACAA CGAGGAGAGC TGCAATAGCT          2200
CAGAGGACGA ATCAAAATGG GTAGAAGAAC TCATGAAGAT          2240
GCACACAGCT AGGGTGCGTG ACATTGAACA TCTCACCAGC          2280
CTGGACTTCT TCCGAAAGAC CAGCCGCAGC TACCCAGAAA          2320
TCCTGACACT CAAGACATAC CTGCATACAT ATGAGAGCGA          2360
GATTTAACTT TCTGAGCATC TGCAGTACAG TCTTATCAAC          2400
TGGTTGTATA TTTTTATATT GTTTTTGTAT TTATTAATTT          2440
GAAACCAGGA CATTAAAAAT GTTAGTATTT TAATCCTGTA          2480
CCAAATCTGA CATATTATGC CTGAATGACT CCACTGTTTT          2520
TCTCTAATGC TTGATTTAGG TAGCCTTGTG TTCTGAGTAG          2560
AGCTTGTAAT AAATACTGCA GCTTGAGTTT TTAGTGGAAG          2600
CTTCTAAATG GTGCTGCAGA TTTGATATTT GCATTGAGGA          2640
AATATTAATT TTCCAATGCA CAGTTGCCAC ATTTAGTCCT          2680
GTACTGTATG GAAACACTGA TTTTGTAAAG TT                  2712
```

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 979
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: No

(vi) ORIGINAL SOURCE:
- (A) ORGANISM: Human
- (B) STRAIN:
- (C) INDIVIDUAL ISOLATE:
- (D) DEVELOPMENTAL STAGE:
- (E) HAPLOTYPE:

(F) TISSUE TYPE: Liver
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: putative autotaxin protein sequence from human liver

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

```
Met Ala Arg Arg Ser Ser Phe Gln Ser Cys Gln Asp
 1               5                  10
Ile Ser Leu Phe Thr Phe Ala Val Gly Val Asn Ile
            15                  20
Cys Leu Gly Phe Thr Ala His Arg Ile Lys Arg Ala
 25                  30                  35
Glu Gly Trp Glu Glu Gly Pro Pro Thr Val Leu Ser
                40                  45
Asp Ser Pro Trp Thr Asn Ile Ser Gly Ser Cys Lys
     50                  55                  60
Gly Arg Cys Phe Glu Leu Gln Glu Ala Gly Pro Pro
                65                  70
Asp Cys Arg Cys Asp Asn Leu Cys Lys Ser Tyr Thr
            75                  80
Ser Cys Cys His Asp Phe Asp Glu Leu Cys Leu Lys
 85                  90                  95
Thr Ala Arg Ala Trp Glu Cys Thr Lys Asp Arg Cys
                100                 105
Gly Glu Val Arg Asn Glu Glu Asn Ala Cys His Cys
    110                 115                 120
Ser Glu Asp Cys Leu Ala Arg Gly Asp Cys Cys Thr
                125                 130
Asn Tyr Gln Val Val Cys Lys Gly Glu Ser His Trp
            135                 140
Val Asp Asp Asp Cys Glu Glu Ile Lys Ala Ala Glu
145                 150                 155
Cys Leu Gln Val Cys Ser Pro Ser Ile Asn His Leu
                160                 165
Leu Arg Gly Trp Leu Pro Met Thr Ser Tyr Met Lys
    170                 175                 180
Lys Gly Ser Lys Val Met Pro Asn Ile Glu Lys Leu
                185                 190
Arg Ser Cys Gly Thr His Ser Pro Tyr Met Arg Pro
            195                 200
Val Tyr Pro Thr Lys Thr Phe Pro Asn Leu Tyr Thr
205                 210                 215
Leu Ala Thr Gly Leu Tyr Pro Glu Ser His Gly Ile
                220                 225
Val Gly Asn Ser Met Tyr Asp Pro Val Phe Asp Ala
    230                 235                 240
```

Thr Phe His Leu Arg Gly Arg Glu Lys Phe Asn His
245 250
Arg Trp Trp Gly Gly Gln Pro Leu Trp Ile Thr Ala
255 260
Thr Lys Gln Arg Gly Glu Ser Trp Asn Ile Leu Leu
265 270 275
Val Cys Cys His Pro Ser Arg Ala Glu Ile Leu Thr
280 285
Ile Leu Gln Trp Leu Thr Leu Pro Asp His Glu Arg
290 295 300
Pro Ser Val Tyr Ala Phe Tyr Ser Glu Gln Pro Asp
305 310
Phe Ser Gly His Lys His Met Pro Phe Gly Pro Glu
315 320
Met Thr Asn Pro Leu Arg Glu Met His Lys Ile Val
325 330 335
Gly Gln Leu Met Asp Gly Leu Lys Gln Leu Lys Leu
340 345
His Arg Cys Val Asn Val Ile Phe Val Glu Thr Met
350 355 360
Asp Gly Arg Cys His Met Tyr Arg Thr Glu Phe Leu
365 370
Ser Asn Tyr Leu Thr Asn Val Asp Asp Ile Thr Leu
375 380
Val Pro Gly Thr Leu Gly Arg Ile Arg Ser Lys Phe
385 390 395
Ser Asn Asn Ala Lys Tyr Asp Pro Lys Ala Ile Ile
400 405
Ala Asn Leu Thr Cys Lys Lys Pro Asp Gln His Phe
410 415 420
Lys Pro Tyr Leu Lys Gln His Leu Pro Lys Arg Leu
425 430
His Tyr Ala Asn Asn Arg Arg Ile Glu Asp Ile His
435 440
Leu Leu Val Glu Arg Arg Trp His Val Ala Arg Lys
445 450 455
Pro Leu Asp Val Tyr Lys Lys Pro Ser Gly Asn Ala
460 465
Phe Ser Arg Glu Thr Thr Ala Phe Asp Asn Lys Val
470 475 480
Asn Ser Met Gln Thr Val Phe Val Gly Tyr Gly Pro
485 490
Thr Phe Lys Tyr Lys Thr Lys Val Pro Pro Phe Glu
495 500
Asn Ile Glu Leu Tyr Asn Val Met Cys Asp Leu Leu
505 510 515
Gly Leu Lys Pro Ala Pro Asn Asn Gly Thr His Gly
520 525
Ser Leu Asn His Leu Leu Arg Thr Asn Thr Phe Arg
530 535 540
Pro Thr Met Pro Glu Glu Val Thr Arg Pro Asn Tyr
545 550
Pro Gly Ile Met Tyr Leu Gln Ser Asp Phe Asp Leu
555 560

Gly Cys Thr Cys Asp Asp Lys Val Glu Pro Lys Asn
565 570 575
Lys Leu Asp Glu Leu Asn Lys Arg Leu His Thr Lys
580 585
Gly Ser Thr Glu Glu Arg His Leu Leu Tyr Gly Asp
590 595 600
Arg Pro Ala Val Leu Tyr Arg Thr Arg Tyr Asp Ile
605 610
Leu Tyr His Thr Asp Phe Glu Ser Gly Tyr Ser Glu
615 620
Ile Phe Leu Met Pro Leu Trp Thr Ser Tyr Thr Val
625 630 635
Ser Lys Gln Ala Glu Val Ser Ser Val Pro Asp His
640 645
Leu Thr Ser Cys Val Arg Pro Asp Val Arg Val Ser
650 655 660
Pro Ser Phe Ser Gln Asn Cys Leu Ala Tyr Lys Asn
665 670
Asp Lys Gln Met Ser Tyr Gly Phe Leu Phe Pro Pro
675 680
Tyr Leu Ser Ser Ser Pro Glu Ala Lys Tyr Asp Ala
685 690 695
Phe Leu Val Thr Asn Met Val Pro Met Tyr Pro Ala
700 705
Phe Lys Arg Val Trp Asn Tyr Phe Gln Arg Val Leu
710 715 720
Val Lys Lys Tyr Ala Ser Glu Arg Asn Gly Val Asn
725 730
Val Ile Ser Gly Pro Ile Phe Asp Tyr Asp Tyr Asp
735 740
Gly Leu His Asp Thr Glu Asp Lys Ile Lys Gln Tyr
745 750 755
Val Glu Gly Ser Ser Ile Pro Val Pro Thr His Tyr
760 765
Tyr Ser Ile Ile Thr Ser Cys Leu Asp Phe Thr Gln
770 775 780
Pro Ala Asp Lys Cys Asp Gly Pro Leu Ser Val Ser
785 790
Ser Phe Ile Leu Pro His Arg Pro Asp Asn Glu Glu
795 800
Ser Cys Asn Ser Ser Glu Asp Glu Ser Lys Trp Val
805 810 815
Glu Glu Leu Met Lys Met His Thr Ala Arg Val Arg
820 825
Asp Ile Glu His Leu Thr Ser Leu Asp Phe Phe Arg
830 835 840
Lys Thr Ser Arg Ser Tyr Pro Glu Ile Leu Thr Leu
845 850
Lys Thr Tyr Leu His Thr Tyr Glu Ser Glu Ile Xaa
855 860
Leu Ser Glu His Leu Gln Tyr Ser Leu Ile Asn Trp
865 870 875
Leu Tyr Ile Phe Ile Leu Phe Leu Tyr Leu Leu Ile
880 885

```
Xaa Asn Gln Asp Ile Lys Asn Val Ser Ile Leu Ile
    890                 895                 900
Leu Tyr Gln Ile Xaa His Ile Met Pro Glu Xaa Leu
                905                 910
His Cys Phe Ser Leu Met Leu Asp Leu Gly Ser Leu
        915                 920
Val Phe Xaa Val Glu Leu Val Ile Asn Thr Ala Ala
925                 930                 935
Xaa Val Phe Ser Gly Ser Phe Xaa Met Val Leu Gln
            940                 945
Ile Xaa Tyr Leu His Xaa Gly Asn Ile Asn Phe Pro
    950                 955                 960
Met His Ser Cys His Ile Xaa Ser Cys Thr Val Trp
                965                 970
Lys His Xaa Phe Cys Lys Val
        975
```

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:8
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: peptide

(iii) HYPOTHETICAL: No

(ix) FEATURE:
(A) NAME/KEY: ATX-204
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

```
Met His Thr Ala Arg Val Arg Asp
                5
```

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

(ix) FEATURE:
(A) NAME/KEY: ATX-205
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Phe Ser Asn Asn Ala Lys Tyr Asp
5

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide

(iii) HYPOTHETICAL: No

(ix) FEATURE:
(A) NAME/KEY: ATX-209
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Val Met Pro Asn Ile Glu Lys
5

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide

(iii) HYPOTHETICAL: No

(ix) FEATURE:
(A) NAME/KEY: ATX-210
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

```
Thr Ala Arg Gly Trp Glu Cys Thr
                5
```

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide

(iii) HYPOTHETICAL: No

(ix) FEATURE:
(A) NAME/KEY: ATX-212
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

```
Xaa Asp Ser Pro Trp Thr Xaa Ile Ser Gly Ser
                5                   10
```

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide

(iii) HYPOTHETICAL: No

(ix) FEATURE:
(A) NAME/KEY: ATX-214
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Leu Arg Ser Cys Gly Thr His Ser Pro Tyr Met
5 10

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide

(iii) HYPOTHETICAL: No

(ix) FEATURE:
(A) NAME/KEY: ATX-215/34A
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Thr Tyr Leu His Thr Tyr Glu Ser
5

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide

(iii) HYPOTHETICAL: No

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Ala Ile Ile Ala Asn Leu Thr Cys Lys Lys Pro Asp Gln
5 10

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide
(iii) HYPOTHETICAL: No

(ix) FEATURE:
(A) NAME/KEY: ATX-216
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Ile Val Gly Gln Leu Met Asp Gly
5

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide

(iii) HYPOTHETICAL: No

(ix) FEATURE:
(A) NAME/KEY: ATX-218/44
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Thr Ser Arg Ser Tyr Pro Glu Ile Leu
5

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9

(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide

(iii) HYPOTHETICAL: No
(ix) FEATURE:
(A) NAME/KEY: ATX-223B/24
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Gln Ala Glu Val Ser Ser Val Pro Asp
5

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide

(iii) HYPOTHETICAL: No

(ix) FEATURE:
(A) NAME/KEY: ATX-224
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Arg Cys Phe Glu Leu Gln Glu Ala Gly Pro Pro Asp Asp Cys
5 10

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(A) DESCRIPTION: Peptide

(iii) HYPOTHETICAL: No

(ix) FEATURE:
(A) NAME/KEY: ATX-229
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Ser Tyr Thr Ser Cys Cys His Asp Phe Asp Glu Leu
5 10

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide

(iii) HYPOTHETICAL: No

(ix) FEATURE:
(A) NAME/KEY: ATX-224/53
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Gln Met Ser Tyr Gly Phe Leu Phe Pro Pro Tyr Leu
1 5 10
Ser Ser Ser Pro
15

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE:
(A) DESCRIPTION: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Human
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE: Liver
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: 5' end of human liver ATX gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

ATGGCAAGGA GGAGCTCGTT CCAGTCGTGT CAAGATATAT 40
CCCTGTTCAC TTTTGCCGTT GGAGTCAATA TCTGCTTAGG 80
ATTCACTGCA CATCGAATTA AGAGAGCAGA AGGATGG 117

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide

(iii) HYPOTHETICAL: No

(v) FRAGMENT TYPE: N-terminal fragment

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Human
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE: Liver
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: N-terminal region including transmembrane domain of liver ATX protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

```
Met Ala Arg Arg Ser Ser Phe Gln Ser Cys Gln Asp
 1               5                  10
Ile Ser Leu Phe Thr Phe Ala Val Gly Val Asn Ile
            15                  20
Cys Leu Gly Phe Thr Ala His Arg Ile Lys Arg Ala
 25                  30                  35
Glu Gly Trp
```

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: Yes

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: Primer from 5' end of 4C11

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

GCTCAGATAA GGAGGAAAGA G 21

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: Yes

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: Nested primers from 4C11

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

GAATCCGTAG GACATCTGCT T 21

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: Yes

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: Nested primers from 4C11

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

TGTAGGCCAA ACAGTTCTGA C 21

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: Nested sense primer deduced from ATX-101, wherein N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

AAYTCNATGC ARACNGTNTT YGTNG 25

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: Nested primer of ATX -101, wherein N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

TTYGTNGGNT AYGGNCCNAC NTTYAA 26

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: Nested primer deduced from ATX-103, wherein N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

AAYTAYCTNA CNAAYGTNGA YGAYAT 26

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: Nested primer deduced from ATX-103, wherein N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

GAYGAYATNA CNCTNGTNCC NGGNAC 26

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: Nested primer deduced from ATX-103, wherein N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

TGYTTYGARY TNCARGARGC NGGNCCNCC 29

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

GCTGTCTTCA AACACAGC 18

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

CTGGTGGCTG TAATCCATAG C 21

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: Primer for 5′ end of N-tera 2D1 sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

CGTGAAGGCA AAGAGAACAC G 21

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3104
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(ix) FEATURE:
(A) NAME/KEY: N-tera 2D1 ATX cDNA
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

AGTGCACTCC GTGAAGGCAA AGAGAACACG CTGCAAAAGG 40
CTTTCCAATA ATCCTCGACA TGGCAAGGAG GAGCTCGTTC 80
CAGTCGTGTC AGATAATATC CCTGTTCACT TTTGCCGTTG 120
GAGTCAATAT CTGCTTAGGA TTCACTGCAC ATCGAATTAA 160
GAGAGCAGAA GGATGGGAGG AAGGTCCTCC TACAGTGCTA 200
TCAGACTCCC CCTGGACCAA CATCTCCGGA TCTTGCAAGG 240
GCAGGTGCTT TGAACTTCAA GAGGCTGGAC CTCCTGATTG 280
TCGCTGTGAC AACTTGTGTA AGAGCTATAC CAGTTGCTGC 320
CATGACTTTG ATGAGCTGTG TTTGAAGACA GCCCGTGCGT 360
GGGAGTGTAC TAAGGACAGA TGTGGAGAAG TCAGAAATGA 400
AGAAAATGCC TGTCACTGCT CAGAGGACTG CTTGGCCAGG 440
GGAGACTGCT GTACCAATTA CCAAGTGGTT TGCAAAGGAG 480
AGTCGCATTG GGTTGATGAT GACTGTGAGG AAATAAAGGC 520
CGCAGAATGC CCTGCAGGGT TTGTTCGCCC TCCATTAATC 560

ATCTTCTCCG TGGATGGCTT CCGTGCATCA TACATGAAGA 600
AAGGCAGCAA AGTCATGCCT AATATTGAAA AACTAAGGTC 640
TTGTGGCACA CACTCGCCCC ACATGAGGCC GGTGTACCCA 680
ACTAAAACCT TTCCTAACTT ATACACTTTG GCCACTGGGC 720
TATATCCAGA ATCACATGGA ATTGTTGGCA ATTCAATGTA 760
TGATCCTGTA TTTGATGCCA CTTTTCATCT GCGAGGGCGA 800
GAGAAATTTA ATCATAGATG GTGGGGAGGT CAACCGCTAT 840
GGATTACAGC CACCAAGCAA AGGGGTGAAA GCTGGAACAT 880
TCTTTTGGTC TGTTGTCATC CCTCACGAGC GGAGATATTA 920
ACCATATTGC AGTGGCTCAC CCTGCCAGAT CATGAGAGGC 960
TTCGGTCTAT GCCTTCTATT CTGAGCAACC TGATTTCTCT 1000
GGACACAAAT ATGCCTTTCG GCCCTGAGAT GACAAATCCT 1040
CTGAGGGAAA TCGACAAAAT TGTGGGGCAA TTAATGGATG 1080
GACTGAAACA ACTAAAACTG CATCGGTGTG TCAACGTCAT 1120
CTTTGTCGGA GACCATGGAA TGGAAGATGT CACATGTGAT 1160
AGAACTGAGT TCTTGAGTAA TTACCTAACT AATGTGGATG 1200
ATATTACTTT AGTGCCTGGA ACTCTAGGAA TTCGATCCAA 1240
ATTTAGCAAC AATGCTAAAT ATGACCCCAA AGCCATTATT 1280
GCCAATCTCA CGTGTAAAAA ACCAGATCAG CACTTTAAGC 1320
CTTACTTGAA ACAGCACCTT CCCAAACGTT TGCACTATGC 1360
CAACAACAGA AGAATTGAGG ATATCCATTT ATTGGTGGAA 1400
CGCAGATGGC ATGTTGCAAG GAAACCTTTG GATGTTTATA 1440
AGAAACCATC AGGAAAATGC TTTTTCCAGG GAGACCACGG 1480
ATTTGATAAC AAGGTCAACA GCATGCAGAC TGTTTTTGTA 1520
GGTTATGGCC CAACATTTAA GTACAAGACT AAAGTGCCTC 1560
CATTTGAAAA CATTGAACTT TACAATGTTA TGTGTGATCT 1600
CCTGGGATTG AAGCCAGCTC CTAATAATGG GACCCATGGA 1640
AGTTTGAATC ATCTCCTGCG CACTAATACC TTCAGGCCAA 1680
CCATGCCAGA GGAAGTTACC AGACCCAATT ATCCAGGGAT 1720
TATGTACCTT CAGTCTGATT TTGACCTGGG CTGCACTTGT 1760
GATGATAAGG TAGAGCCAAA GAACAAGTTG GATGAACTCA 1800
ACAAACGGCT TCATACAAAA GGGTCTACAG AAGAGAGACA 1840
CCTCCTCTAT GGGCGACCTG CAGTGCTTTA TCGGACTAGA 1880
TATGATGTCT TATATCACAC TGACTTTGAA AGTGGTTATA 1920
GTGAAATATT CCTAATGCCA CTCTGGACAT CATATACTGT 1960
TTCCAAACAG GCTGAGGTTT CCAGCGTTCC TGACCATCTG 2000
ACCAGTTGCG TCCGGCCTGA TGTCCGTGTT TCTCCGAGTT 2040
TCAGTCAGAA CTGTTTGGCC TACAAAAATG ATAAGCAGAT 2080
GTCCTACGGA TTCCTCTTTC CTCCTTATCT GAGCTCTTCA 2120
CCAGAGGCTA AATATGATGC ATTCCTTGTA ACCAATATGG 2160
TTCCAATGTA TCCTGCTTTC AAACGGGTCT GGAATTATTT 2200
CCAAAGGGTA TTGGTGAAGA AATATGCTTC GGAAAGAAAT 2240
GGAGTTAACG TGATAAGTGG ACCAATCTTC GACTATGACT 2280
ATGATGGCTT ACATGACACA GAAGACAAAA TAAAACAGTA 2320
CGTGGAAGGC AGTTCCATTC CTGTTCCAAC TCACTACTAC 2360
AGCATCATCA CCAGCTGTCT GGATTTCACT CAGCCTGCCG 2400
ACAAGTGTGA CGGCCCTCTC TCTGTGTCCT CCTTCATCCT 2440
CCGTCACCGG CCTGACAACG AGGAGAGCTG CAATAGCTCA 2480
GAGGACGAAT CAAAATGGGT AGAAGAACTC ATGAAGATGC 2520
ACACGGCTAG GGTGCGTGAC ATTGAACATC TCACCAGCCT 2560
GGACTTCTTC CGAAAGACCA GCCGCAGCTA CCCAGAAATC 2600
CTGACACTCA AGACATACCT GCATACATAT GAGAGCGAGA 2640
TTTAACTTTC TGAGCATCTG CAGTACAGTC TTATCAACTG 2680
GTTGTATATT TTTATATTGT TTTTGTATTT ATTAATTTGA 2720

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AACCAGGACA TTAAAAATGT TAGTATTTTA ATCCTGTACC          2760
AAATCTGACA TATTATGCCT GAATGACTCC ACTGTTTTTC          2800
TCTAATGCTT GATTTAGGTA GCCTTGTGTT CTGAGTAGAG          2840
CTTGTAATAA ATACTGCAGC TTGAGTTTTT AGTGGAAGCT          2880
TCTAAATGGT GCTGCAGATT TGATATTTGC ATTGAGGAAA          2920
TATTAATTTT CCAATGCACA GTTGCCACAT TTAGTCCTGT          2960
ACTGTATGGA AACACTGATT TTGTAAAGTT GCCTTTATTT          3000
GCTGTTAACT GTTAACTATG ACAGATATAT TTAAGCCTTA          3040
TAAACCAATC TTAAACATAA TAAATCACAC ATTCAGTTTT          3080
TTCTGGTAAA AAAAAAAAAA AAAA                          3104
```

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 861
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: No

(ix) FEATURE:
(A) NAME/KEY: N-tera 2D1 ATX protein
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

```
Met Ala Arg Arg Ser Ser Phe Gln Ser Cys Gln Ile Ile Ser Leu Phe
 1               5                  10                  15
Thr Phe Ala Val Gly Val Asn Ile Cys Leu Gly Phe Thr Ala His Arg
            20                  25                  30
Ile Lys Arg Ala Glu Gly Trp Glu Glu Gly Pro Pro Thr Val Leu Ser
        35                  40                  45
Asp Ser Pro Trp Thr Asn Ile Ser Gly Ser Cys Lys Gly Arg Cys Phe
    50                  55                  60
Glu Leu Gln Glu Ala Gly Pro Pro Asp Cys Arg Cys Asp Asn Leu Cys
65                  70                  75                  80
Lys Ser Tyr Thr Ser Cys Cys His Asp Phe Asp Glu Leu Cys Leu Lys
                85                  90                  95
Thr Ala Arg Ala Trp Glu Cys Thr Lys Asp Arg Cys Gly Glu Val Arg
            100                 105                 110
Asn Glu Glu Asn Ala Cys His Cys Ser Glu Asp Cys Leu Ala Arg Gly
        115                 120                 125
Asp Cys Cys Thr Asn Tyr Gln Val Val Cys Lys Gly Glu Ser His Trp
    130                 135                 140
Val Asp Asp Asp Cys Glu Glu Ile Lys Ala Ala Glu Cys Pro Ala Gly
145                 150                 155                 160
```

Phe Val Arg Pro Pro Leu Ile Ile Phe Ser Val Asp Gly Phe Arg Ala
165 170 175
Ser Tyr Met Lys Lys Gly Ser Lys Val Met Pro Asn Ile Glu Lys Leu
180 185 190
Arg Ser Cys Gly Thr His Ser Pro His Met Arg Pro Val Tyr Pro Thr
195 200 205
Lys Thr Phe Pro Asn Leu Tyr Thr Leu Ala Thr Gly Leu Tyr Pro Glu
210 215 220
Ser His Gly Ile Val Gly Asn Ser Met Tyr Asp Pro Val Phe Asp Ala
225 230 235 240
Thr Phe His Leu Arg Gly Arg Glu Lys Phe Asn His Arg Trp Trp Gly
245 250 255
Gly Gln Pro Leu Trp Ile Thr Ala Thr Lys Gln Arg Gly Glu Ser Trp
260 265 270
Asn Ile Leu Leu Val Cys Cys His Pro Ser Arg Ala Glu Ile Leu Thr
275 280 285
Ile Leu Gln Trp Leu Thr Leu Pro Asp His Glu Arg Leu Arg Ser Met
290 295 300
Pro Ser Ile Leu Ser Asn Leu Ile Ser Leu Asp Thr Asn Met Pro Phe
305 310 315 320
Gly Pro Glu Met Thr Asn Pro Leu Arg Glu Ile Asp Lys Ile Val Gly
325 330 335
Gln Leu Met Asp Gly Leu Lys Gln Leu Lys Leu His Arg Cys Val Asn
340 345 350
Val Ile Phe Val Gly Asp His Gly Met Glu Asp Val Thr Cys Asp Arg
355 360 365
Thr Glu Phe Leu Ser Asn Tyr Leu Thr Asn Val Asp Asp Ile Thr Leu
370 375 380
Val Pro Gly Thr Leu Gly Ile Arg Ser Lys Phe Ser Asn Asn Ala Lys
385 390 395 400
Tyr Asp Pro Lys Ala Ile Ile Ala Asn Leu Thr Cys Lys Lys Pro Asp
405 410 415
Gln His Phe Lys Pro Tyr Leu Lys Gln His Leu Pro Lys Arg Leu His
420 425 430
Tyr Ala Asn Asn Arg Arg Ile Glu Asp Ile His Leu Leu Val Glu Arg
435 440 445
Arg Trp His Val Ala Arg Lys Pro Leu Asp Val Tyr Lys Lys Pro Ser
450 455 460
Gly Lys Cys Phe Phe Gln Gly Asp His Gly Phe Asp Asn Lys Val Asn
465 470 475 480
Ser Met Gln Thr Val Phe Val Gly Tyr Gly Pro Thr Phe Lys Tyr Lys
485 490 495
Thr Lys Val Pro Pro Phe Glu Asn Ile Glu Leu Tyr Asn Val Met Cys
500 505 510
Asp Leu Leu Gly Leu Lys Pro Ala Pro Asn Asn Gly Thr His Gly Ser
515 520 525
Leu Asn His Leu Leu Arg Thr Asn Thr Phe Arg Pro Thr Met Pro Glu
530 535 540
Glu Val Thr Arg Pro Asn Tyr Pro Gly Ile Met Tyr Leu Gln Ser Asp
445 450 555 560
Phe Asp Leu Gly Cys Thr Cys Asp Asp Lys Val Glu Pro Lys Asn Lys
565 570 575
Leu Asp Glu Leu Asn Lys Arg Leu His Thr Lys Gly Ser Thr Glu Glu
580 585 590

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Arg His Leu Leu Tyr Gly Arg Pro Ala Val Leu Tyr Arg Thr Arg Tyr
        595                 600                 605
Asp Val Leu Tyr His Thr Asp Phe Glu Ser Gly Tyr Ser Glu Ile Phe
    610                 615                 620
Leu Met Pro Leu Trp Thr Ser Tyr Thr Val Ser Lys Gln Ala Glu Val
625                 630                 635                 640
Ser Ser Val Pro Asp His Leu Thr Ser Cys Val Arg Pro Asp Val Arg
                645                 650                 655
Val Ser Pro Ser Phe Ser Gln Asn Cys Leu Ala Tyr Lys Asn Asp Lys
            660                 665                 670
Gln Met Ser Tyr Gly Phe Leu Phe Pro Pro Tyr Leu Ser Ser Ser Pro
        675                 680                 685
Glu Ala Lys Tyr Asp Ala Phe Leu Val Thr Asn Met Val Pro Met Tyr
    690                 695                 700
Pro Ala Phe Lys Arg Val Trp Asn Tyr Phe Gln Arg Val Leu Val Lys
705                 710                 715                 720
Lys Tyr Ala Ser Glu Arg Asn Gly Val Asn Val Ile Ser Gly Pro Ile
                725                 730                 735
Phe Asp Tyr Asp Tyr Asp Gly Leu His Asp Thr Glu Asp Lys Ile Lys
            740                 745                 750
Gln Tyr Val Glu Gly Ser Ser Ile Pro Val Pro Thr His Tyr Tyr Ser
        755                 760                 765
Ile Ile Thr Ser Cys Leu Asp Phe Thr Gln Pro Ala Asp Lys Cys Asp
    770                 775                 780
Gly Pro Leu Ser Val Ser Ser Phe Ile Leu Arg His Arg Pro Asp Asn
785                 790                 795                 800
Glu Glu Ser Cys Asn Ser Ser Glu Asp Glu Ser Lys Trp Val Glu Glu
                805                 810                 815
Leu Met Lys Met His Thr Ala Arg Val Arg Asp Ile Glu His Leu Thr
            820                 825                 830
Ser Leu Asp Phe Phe Arg Lys Thr Ser Arg Ser Tyr Pro Glu Ile Leu
        835                 840                 845
Thr Leu Lys Thr Tyr Leu His Thr Tyr Glu Ser Glu Ile
    850                 855                 860
```

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3251
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(ix) FEATURE:
(A) NAME/KEY: A2058 ATX cDNA
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

```
CGTGAAGGCA AAGAGAACAC GCTGCAAAAG GCTTCCAAGA                40
ATCCTCGACA TGGCAAGGAG GAGCTCGTTC CAGTCGTGTC                80
AGATAATATC CCTGTTCACT TTTGCCGTTG GAGTCAGTAT               120
CTGCTTAGGA TTCACTGCAC ATCGAATTAA GAGAGCAGAA               160
GGATGGGAGG AAGGTCCTCC TACAGTGCTA TCAGACTCCC               200
CCTGGACCAA CATCTCCGGA TCTTGCAAGG GCAGGTGCTT               240
TGAACTTCAA GAGGCTGGAC CTCCTGATTG TCGCTGTGAC               280
AACTTGTGTA AGAGCTATAC CAGTTGCTGC CATGACTTTG               320
ATGAGCTGTG TTTGAAGACA GCCCGTGGCT GGGAGTGTAC               360
TAAGGACAGA TGTGGAGAAG TCAGAAATGA AGAAAATGCC               400
TGTCACTGCT CAGAGGACTG CTTGGCCAGG GGAGACTGCT               440
GTACCAATTA CCAAGTGGTT TGCAAAGGAG AGTCGCATTG               480
GGTTGATGAT GACTGTGAGG AAATAAAGGC CGCAGAATGC               520
CCTGCAGGGT TTGTTCGCCC TCCATTAATC ATCTTCTCCG               560
TGGATGGCTT CCGTGCATCA TACATGAAGA AAGGCAGCAA               600
AGTCATGCCT AATATTGAAA AACTAAGGTC TTGTGGCACA               640
CACTCTCCCT ACATGAGGCC GGTGTACCCA ACTAAAACCT               680
TTCCTAACTT ATACACTTTG GCCACTGGGC TATATCCAGA               720
ATCACATGGA ATTGTTGGCA ATTCAATGTA TGATCCTGTA               760
TTTGATGCCA CTTTTCATCT GCGAGGGCGA GAGAAATTTA               800
ATCATAGATG GTGGGGAGGT CAACCGCTAT GGATTACAGC               840
CACCAAGCAA GGGGTGAAAG CTGGAACATT CTTTTGGTCT               880
GTTGTCATCC CTCACGAGCG GAGAATATTA ACCATATTGC               920
GGTGGCTCAC CCTGCCAGAT CATGAGAGGC CTTCGGTCTA               960
TGCCTTCTAT TCTGAGCAAC CTGATTTCTC TGGACACAAA              1000
TATGGCCCTT TCGGCCCTGA GGAGAGTAGT TATGGCTCAC              1040
CTTTTACTCC GGCTAAGAGA CCTAAGAGGA AAGTTGCCCC              1080
TAAGAGGAGA CAGGAAAGAC CAGTTGCTCC TCCAAAGAAA              1120
AGAAGAAGAA AAATACATAG GATGGATCAT TATGCTGCGG              1160
AAACTCGTCA GGACAAAATG ACAAATCCTC TGAGGGAAAT              1200
CGACAAAATT GTGGGGCAAT TAATGGATGG ACTGAAACAA              1240
CTAAAACTGC GTCGGTGTGT CAACGTCATC TTTGTCGGAG              1280
ACCATGGAAT GGAAGATGTC ACATGTGATA GAACTGAGTT              1320
CTTGAGTAAT TACCTAACTA ATGTGGATGA TATTACTTTA              1360
GTGCCTGGAA CTCTAGGAAG AATTCGATCC AAATTTAGCA              1400
ACAATGCTAA ATATGACCCC AAAGCCATTA TTGCCAATCT              1440
CACGTGTAAA AAACCAGATC AGCACTTTAA GCCTTACTTG              1480
AAACAGCACC TTCCCAAACG TTTGCACTAT GCCAACAACA              1520
GAAGAATTGA GGATATCCAT TTATTGGTGG AACGCAGATG              1560
GCATGTTGCA AGGAAACCTT TGGATGTTTA TAAGAAACCA              1600
TCAGGAAAAT GCTTTTTCCA GGGAGACCAC GGATTTGATA              1640
ACAAGGTCAA CAGCATGCAG ACTGTTTTTG TAGGTTATGG              1680
CCCAACATTT AAGTACAAGA CTAAAGTGCC TCCATTTGAA              1720
AACATTGAAC TTTACAATGT TATGTGTGAT CTCCTGGGAT              1760
TGAAGCCAGC TCCTAATAAT GGGACCCATG GAAGTTTGAA              1800
TCATCTCCTG CGCACTAATA CCTTCAGGCC AACCATGCCA              1840
GAGGAAGTTA CCAGACCCAA TTATCCAGGG ATTATGTACC              1880
TTCAGTCTGA TTTTGACCTG GGCTGCACTT GTGATGATAA              1920
GGTAGAGCCA AAGAACAAGT TGGATGAACT CAACAAACGG              1960
CTTCATACAA AAGGGTCTAC AGAAGAGAGA CACCTCCTCT              2000
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ATGGGCGACC TGCAGTGCTT TATCGGACTA GATATGATAT          2040
CTTATATCAC ACTGACTTTG AAAGTGGTTA TAGTGAAATA          2080
TTCCTAATGC TACTCTGGAC ATCATATACT GTTTCCAAAC          2120
AGGCTGAGGT TTCCAGCGTT CCTGACCATC TGACCAGTTG          2160
CGTCCGGCCT GATGTCCGTG TTTCTCCGAG TTTCAGTCAG          2200
AACTGTTTGG CCTACAAAAA TGATAAGCAG ATGTCCTACG          2240
GATTCCTCTT TCCTCCTTAT CTGAGCTCTT CACCAGAGGC          2280
TAAATATGAT GCATTCCTTG TAACCAATAT GGTTCCAATG          2320
TATCCTGCTT TCAAACGGGT CTGGAATTAT TTCCAAAGGG          2360
TATTGGTGAA GAAATATGCT TCGGAAAGAA ATGGAGTTAA          2400
CGTGATAAGT GGACCAATCT TCGACTATGA CTATGATGGC          2440
TTACATGACA CAGAAGACAA AATAAAACAG TACGTGGAAG          2480
GCAGTTCCAT TCCTGTTCCA ACTCACTACT ACAGCATCAT          2520
CACCAGCTGT CTGGATTTCA CTCAGCCTGC CGACAAGTGT          2560
GACGGCCCTC TCTCTGTGTC CTCCTTCATC CTGCCTCACC          2600
GGCCTGACAA CGAGGAGAGC TGCAATAGCT CAGAGGACGA          2640
ATCAAAATGG GTAGAAGAAC TCATGAAGAT GCACACAGCT          2680
AGGGTGCGTG ACATTGAACA TCTCACCAGC CTGGACTTCT          2720
TCCGAAAGAC CAGCCGCAGC TACCCAGAAA TCCTGACACT          2760
CAAGACATAC CTGCATACAT ATGAGAGCGA GATTTAACTT          2800
TCTGAGCATC TGCAGTACAG TCTTATCAAC TGGTTGTATA          2840
TTTTTATATT GTTTTTGTAT TTATTAATTT GAAACCAGGA          2880
CATTAAAAAT GTTAGTATTT TAATCCTGTA CCAAATCTGA          2920
CATATTATGC CTGAATGACT CCACTGTTTT TCTCTAATGC          2960
TTGATTTAGG TAGCCTTGTG TTCTGAGTAG AGCTTGTAAT          3000
AAATACTGCA GCTTGAGAAA AAGTGGAAGC TTCTAAATGG          3040
TGCTGCAGAT TTGATATTTG CATTGAGGAA ATATTAATTT          3080
TCCAATGCAC AGTTGCCACA TTTAGTCCTG TACTGTATGG          3120
AAACACTGAT TTTGTAAAGT TGCCTTTATT TGCTGTTAAC          3160
TGTTAACTAT GACAGATATA TTTAAGCCTT ATAAACCAAT          3200
CTTAAACATA ATAAATCACA CATTCAGTTT TAAAAAAAAA          3240
AAAAAAAAAA A                                        3251
```

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 915
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(ix) FEATURE:
- (A) NAME/KEY: A2058 ATX protein
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

```
Met Ala Arg Arg Ser Ser Phe Gln Ser Cys Gln Ile
 1               5                  10
Ile Ser Leu Phe Thr Phe Ala Val Gly Val Ser Ile
            15                  20
Cys Leu Gly Phe Thr Ala His Arg Ile Lys Arg Ala
 25                  30                  35
Glu Gly Trp Glu Glu Gly Pro Pro Thr Val Leu Ser
             40                  45
Asp Ser Pro Trp Thr Asn Ile Ser Gly Ser Cys Lys
     50                  55                  60
Gly Arg Cys Phe Glu Leu Gln Glu Ala Gly Pro Pro
                 65                  70
Asp Cys Arg Cys Asp Asn Leu Cys Lys Ser Tyr Thr
            75                  80
Ser Cys Cys His Asp Phe Asp Glu Leu Cys Leu Lys
 85                  90                  95
Thr Ala Arg Gly Trp Glu Cys Thr Lys Asp Arg Cys
             100                 105
Gly Glu Val Arg Asn Glu Glu Asn Ala Cys His Cys
    110                 115                 120
Ser Glu Asp Cys Leu Ala Arg Gly Asp Cys Cys Thr
                125                 130
Asn Tyr Gln Val Val Cys Lys Gly Glu Ser His Trp
            135                 140
Val Asp Asp Asp Cys Glu Glu Ile Lys Ala Ala Glu
145                 150                 155
Cys Pro Ala Gly Phe Val Arg Pro Pro Leu Ile Ile
             160                 165
Phe Ser Val Asp Gly Phe Arg Ala Ser Tyr Met Lys
    170                 175                 180
Lys Gly Ser Lys Val Met Pro Asn Ile Glu Lys Leu
                185                 190
Arg Ser Cys Gly Thr His Ser Pro Tyr Met Arg Pro
            195                 200
Val Tyr Pro Thr Lys Thr Phe Pro Asn Leu Tyr Thr
205                 210                 215
Leu Ala Thr Gly Leu Tyr Pro Glu Ser His Gly Ile
             220                 225
Val Gly Asn Ser Met Tyr Asp Pro Val Phe Asp Ala
    230                 235                 240
Thr Phe His Leu Arg Gly Arg Glu Lys Phe Asn His
                245                 250
Arg Trp Trp Gly Gly Gln Pro Leu Trp Ile Thr Ala
            255                 260
Thr Lys Gln Gly Val Lys Ala Gly Thr Phe Phe Trp
265                 270                 275
Ser Val Val Ile Pro His Glu Arg Arg Ile Leu Thr
             280                 285
Ile Leu Arg Trp Leu Thr Leu Pro Asp His Glu Arg
    290                 295                 300
Pro Ser Val Tyr Ala Phe Tyr Ser Glu Gln Pro Asp
                305                 310
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Phe Ser Gly His Lys Tyr Gly Pro Phe Gly Pro Glu
        315                 320
Glu Ser Ser Tyr Gly Ser Pro Phe Thr Pro Ala Lys
325                 330                 335
Arg Pro Lys Arg Lys Val Ala Pro Lys Arg Arg Gln
            340                 345
Glu Arg Pro Val Ala Pro Pro Lys Lys Arg Arg Arg
    350                 355                 360
Lys Ile His Arg Met Asp His Tyr Ala Ala Glu Thr
                365                 370
Arg Gln Asp Lys Met Thr Asn Pro Leu Arg Glu Ile
        375                 380
Asp Lys Ile Val Gly Gln Leu Met Asp Gly Leu Lys
385                 390                 395
Gln Leu Lys Leu Arg Arg Cys Val Asn Val Ile Phe
            400                 405
Val Gly Asp His Gly Met Glu Asp Val Thr Cys Asp
    410                 415                 420
Arg Thr Glu Phe Leu Ser Asn Tyr Leu Thr Asn Val
                425                 430
Asp Asp Ile Thr Leu Val Pro Gly Thr Leu Gly Arg
        435                 440
Ile Arg Ser Lys Phe Ser Asn Asn Ala Lys Tyr Asp
445                 450                 455
Pro Lys Ala Ile Ile Ala Asn Leu Thr Cys Lys Lys
            460                 465
Pro Asp Gln His Phe Lys Pro Tyr Leu Lys Gln His
    470                 475                 480
Leu Pro Lys Arg Leu His Tyr Ala Asn Asn Arg Arg
                485                 490
Ile Glu Asp Ile His Leu Leu Val Glu Arg Arg Trp
        495                 500
His Val Ala Arg Lys Pro Leu Asp Val Tyr Lys Lys
505                 510                 515
Pro Ser Gly Lys Cys Phe Phe Gln Gly Asp His Gly
            520                 525
Phe Asp Asn Lys Val Asn Ser Met Gln Thr Val Phe
    530                 535                 540
Val Gly Tyr Gly Pro Thr Phe Lys Tyr Lys Thr Lys
                545                 550
Val Pro Pro Phe Glu Asn Ile Glu Leu Tyr Asn Val
        555                 560
Met Cys Asp Leu Leu Gly Leu Lys Pro Ala Pro Asn
565                 570                 575
Asn Gly Thr His Gly Ser Leu Asn His Leu Leu Arg
            580                 585
Thr Asn Thr Phe Arg Pro Thr Met Pro Glu Glu Val
    590                 595                 600
Thr Arg Pro Asn Tyr Pro Gly Ile Met Tyr Leu Gln
                605                 610
Ser Asp Phe Asp Leu Gly Cys Thr Cys Asp Asp Lys
        615                 620
Val Glu Pro Lys Asn Lys Leu Asp Glu Leu Asn Lys
625                 630                 635
```

Arg Leu His Thr Lys Gly Ser Thr Glu Glu Arg His
640 645
Leu Leu Tyr Gly Arg Pro Ala Val Leu Tyr Arg Thr
650 655 660
Arg Tyr Asp Ile Leu Tyr His Thr Asp Phe Glu Ser
665 670
Gly Tyr Ser Glu Ile Phe Leu Met Leu Leu Trp Thr
675 680
Ser Tyr Thr Val Ser Lys Gln Ala Glu Val Ser Ser
685 690 695
Val Pro Asp His Leu Thr Ser Cys Val Arg Pro Asp
700 705
Val Arg Val Ser Pro Ser Phe Ser Gln Asn Cys Leu
710 715 720
Ala Tyr Lys Asn Asp Lys Gln Met Ser Tyr Gly Phe
725 730
Leu Phe Pro Pro Tyr Leu Ser Ser Ser Pro Glu Ala
735 740
Lys Tyr Asp Ala Phe Leu Val Thr Asn Met Val Pro
745 750 755
Met Tyr Pro Ala Phe Lys Arg Val Trp Asn Tyr Phe
760 765
Gln Arg Val Leu Val Lys Lys Tyr Ala Ser Glu Arg
770 775 780
Asn Gly Val Asn Val Ile Ser Gly Pro Ile Phe Asp
785 790
Tyr Asp Tyr Asp Gly Leu His Asp Thr Glu Asp Lys
795 800
Ile Lys Gln Tyr Val Glu Gly Ser Ser Ile Pro Val
805 810 815
Pro Thr His Tyr Tyr Ser Ile Ile Thr Ser Cys Leu
820 825
Asp Phe Thr Gln Pro Ala Asp Lys Cys Asp Gly Pro
830 835 840
Leu Ser Val Ser Ser Phe Ile Leu Pro His Arg Pro
845 850
Asp Asn Glu Glu Ser Cys Asn Ser Ser Glu Asp Glu
855 860
Ser Lys Trp Val Glu Glu Leu Met Lys Met His Thr
865 870 875
Ala Arg Val Arg Asp Ile Glu His Leu Thr Ser Leu
880 885
Asp Phe Phe Arg Lys Thr Ser Arg Ser Tyr Pro Glu
890 895 900
Ile Leu Thr Leu Lys Thr Tyr Leu His Thr Tyr
905 910
Glu Ser Glu Ile
916